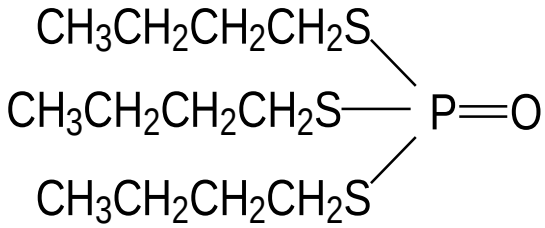
EVALUATION OF S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE (DEF) AS A TOXIC AIR CONTAMINANT



Part C

Human Health Assessment

California Department of Pesticide Regulation
Medical Toxicology Branch

California Environmental Protection Agency
Sacramento, California

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California Environmental Protection Agency
Department of Pesticide Regulation

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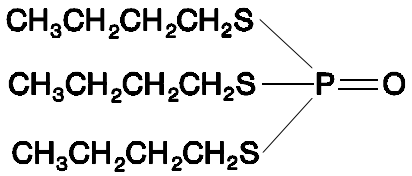

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I. SUMMARY

The only registered use of S,S,S-tributyl phosphorotrithioate (DEF) has been as a cotton defoliant. n-Butyl mercaptan (nBM) is a volatile degradation product of DEF that has a strong skunk-like odor. nBM is produced both during the manufacture of DEF formulations and after application of DEF in the environment. The odor threshold for DEF in humans is approximately 0.01 to 0.1 ppb. Due to public concerns about the odor associated with the use of DEF, the concentration of nBM in formulations of DEF sold or used in California was limited to less than 0.1%. Despite the use of low-odor formulations, complaints from residents have continued. Public health concerns were also raised because of evidence that DEF caused delayed neuropathy. This health assessment addresses the potential health effects from exposure of the general public to DEF in ambient air due to its use as a cotton defoliant.

Several metabolic pathways have been proposed for DEF based on a few metabolites; however, the metabolism of DEF by the various routes of exposure is still highly speculative. DEF sulfoxide and S,S-dibutyl-S-1-hydroxybutyl phosphorotrithioate were identified in rat urine after intraperitoneal injection of DEF. A number of metabolites were detected in the urine and feces of several species (rat, goat, chicken) after oral administration of DEF; however, only one metabolite, butyl-gamma-glutamylcysteinylglycine, was identified in rat urine. One explanation for the inability to identify metabolites was that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. nBM was identified in the excreta of hens administered DEF orally. It was proposed that DEF was hydrolyzed to nBM in the gut causing the hematological effects which were only observed with oral administration of DEF. Apparently nBM inhibits glucose-6-phosphate dehydrogenase leading ultimately to red blood cell lysis through the formation of methemoglobin and Heinz bodies. nBM is also thought to be a product of the normal metabolism of DEF in tissues.

The acute adverse health effects of DEF in experimental animals are due primarily to its inhibition of various esterases including cholinesterase (ChE) and neuropathy target esterase. The clinical signs observed include both cholinergic signs and delayed neuropathy. Hematological changes were also seen with acute exposure to DEF. Although the technical grade DEF was only mildly irritating to the eyes and skin, the DEF 6 formulation is corrosive to the skin and is a severe eye irritant. The no-observed-adverse-effect level (NOAEL) selected by DPR toxicologists for evaluating the acute exposure to DEF in ambient air was 12.2 mg/m^3 (2.9 mg/kg) based on reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, increased startle response observed at 59.5 mg/m^3 (14.3 mg/kg) after 1-3 days of exposure for 6 hours/day. DPR toxicologists generally have not considered plasma and erythrocyte ChE

inhibition in the absence of clinical signs and symptoms to be an adverse effect because the ChEs in blood have no known physiological function. However, the Scientific Review Panel for Toxic Air Contaminants recommended that blood ChE inhibition be considered an adverse effect due to the possible role of plasma ChE in the metabolism of various drugs. The acute NOAEL for blood ChE inhibition for this study was 2.4 mg/m^3 (0.6 mg/kg) based on reduced plasma and erythrocyte ChE activity during the first week of this 13-week study.

The neurological effects were also the predominant adverse effects seen with subchronic exposure, although reduced electroretinographic (ERG) responses, pale retinal fundus and fatty droplets in the adrenal glands were observed at the same dose level as neurological effects in one rat inhalation study. In a rat reproductive toxicity study, several reproductive effects were observed including reduced fertility, birth, and viability indices, increased gestation length, reduced pup weights, cannibalism of pups, and discolored pup livers. The NOAEL selected by DPR toxicologists for evaluating seasonal exposure to DEF in ambient air was also 12.2 mg/m^3 (2.9 mg/kg/day) based on clinical signs, brain cholinesterase (ChE) inhibition, hematological changes, impaired retinal function, pale retinal fundus, fatty droplets in the adrenal glands, and increased adrenal gland weights in rats exposed to DEF in air at 59.5 mg/m^3 (14.3 mg/kg/day) for 6 hours/day, 5 days/week for 13 weeks. The subchronic NOAEL for blood ChE inhibition for this study was 2.4 mg/m^3 (0.6 mg/kg/day) based on reduced plasma and erythrocyte ChE activity during weeks 4-13.

No chronic inhalation studies were available for DEF. Several chronic feeding studies for DEF were available in which hematological changes, brain ChE inhibition, reduced weight gain, and transient hypothermia were observed in rats, mice and/or dogs. There were also dose-related increases in numerous non-neoplastic lesions in the gastrointestinal tract (dilated/distended small intestine and cecum, small intestine vacuolar degeneration, rectal necrosis/ulceration), liver (hypertrophy), adrenal glands (degeneration/pigmentation), and spleen (hematopoiesis) of mice. Dose-related increases in several pre-neoplastic lesions were seen in the small intestine (mucosal hyperplasia and focal atypia) and lung (focal hyperplasia and epithelialization) of mice. In rats, histological changes in the small intestine (hyperplasia and vacuolar degeneration), liver (cytoplasmic vacuolation), and adrenal glands (vacuolar degeneration) were also observed. In addition, numerous ocular effects were observed in one rat study at the highest dose including corneal opacity, lens opacity, cataracts, corneal neovascularization, iritis, uveitis, bilateral flat ERG responses, bilateral retinal atrophy, and optical nerve atrophy. A chronic NOAEL was not selected for evaluating the long-term exposure in humans to DEF in ambient air because the effects seen in the chronic studies were either addressed in evaluating the seasonal exposure (brain ChE inhibition, hematological changes, lesions in the eye and adrenal glands) or the effects

were not considered relevant to the human exposure scenario because the differences in the duration or route of exposure (lesions in the small intestine, liver or spleen).

The available genotoxicity data for DEF were negative. There was also no evidence of oncogenicity in a 2-year rat study for DEF. However, there was an increase in adenocarcinomas of the small intestine (both sexes), liver hemangiosarcomas (males), and alveolar/bronchiolar adenomas (females) in a 90-week mouse oncogenicity study. The increase in small intestine adenocarcinomas and the alveolar/bronchiolar adenomas was only significant at the highest dose (250 ppm). On the other hand, the increase in liver hemangiosarcomas was seen in both the mid- and high-dose males (50 and 250 ppm), although it was only statistically significant at 250 ppm. Although the evidence of oncogenicity was limited to one species and one study, multiple sites were involved in both sexes. Furthermore, small intestine adenocarcinoma is a very rare tumor type. Consequently, the potential oncogenic risk to humans was evaluated using a linear, low dose extrapolation model to estimate potency. Based on the incidence of liver hemangiosarcomas in male mice, the estimated oncogenic potency of DEF ranged from 3.3×10^{-2} (maximum likelihood estimate) to 5.9×10^{-2} (95% upper bound) $(mg/kg/day)^{-1}$. After correcting for oral absorption, the adjusted oncogenic potency ranged from 4.7×10^{-2} to 8.4×10^{-2} $(mg/kg/day)^{-1}$.

Only limited toxicity data were available for nBM. Some clinical signs observed in animals after acute exposure to nBM were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma. Other clinical signs observed with acute exposure included restlessness, increased respiration, diarrhea (oral exposure), sneezing (inhalation exposure), and ocular irritation. Histopathological lesions were observed in the liver (lymphatic infiltration and necrotic foci with small hemorrhages), and kidney (cloudy swelling of the tubules and hyaline casts in the lumina) with all routes of exposure. With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage were also seen. In an inhalation developmental toxicity study, maternal and developmental effects were observed in mice including increased mortality, reduced body weight gain, clinical signs in the dams, increased post-implantation loss, and fetal malformations with a NOAEL of 10 ppm (17 mg/kg/day). No subchronic, reproductive toxicity, chronic toxicity/oncogenicity or genotoxicity data were available for nBM.

Exposure estimates were not calculated from the off-site (application) monitoring data because the monitoring sites in the available studies were all within the buffer zone. Exposure estimates were calculated using the ambient air monitoring from two different cotton growing regions in the San Joaquin Valley. The Five Points monitoring site in Fresno county was selected for initial calculations since it had the highest air concentrations of DEF for both a single day and

on average over the 60-day cotton defoliation season. The assumption was made that if exposures were acceptable at this location they would also be acceptable at the other three Fresno county monitoring sites. The estimated human acute exposure dosages or absorbed daily dosages (ADDs) for DEF at Five Points ranged from 94.2 to 303.5 ng/kg. The seasonal average daily dosages (SADDs) at Five Points ranged from 38.1 to 122.7 ng/kg/day. The annual average daily dosages (AADDs) at Five Points ranged from 6.3 to 20.2 ng/kg/day. Ambient air monitoring data from six rural locations in Kern county was also examined. Since fewer air samples were collected in this study, samples from all six locations were combined. The ADDs for ambient air in Kern county ranged from 16.2 to 52.2 ng/kg. The SADDs ranged from 7.1 to 22.8 ng/kg/day. The AADDs ranged from 1.2 to 3.7 ng/kg/day. Due to their higher respiratory rate relative to their body weight, children consistently had the highest exposure estimates. Air concentrations of nBM were monitored in only one study that lacked sufficient detail to calculate reliable exposure dosages.

The risk for acute and subchronic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOAEL from experimental animal studies to the human exposure dosage. In general, a MOE of at least 100 is desirable to account for interspecies and intraspecies variation in susceptibility. The estimated acute MOEs for DEF in ambient air were equal to or greater than 2,000 when the NOAEL for blood ChE inhibition was used. The acute MOEs were all greater than 9,000 when the NOAEL for clinical signs was used. The seasonal MOEs for DEF in ambient air were greater than 4,000 based on the NOAEL for blood ChE inhibition. Using the NOAEL for more overt subchronic effects, the seasonal MOEs are greater than 20,000. Chronic MOEs were not calculated because exposure was clearly limited to 60-day season during cotton defoliation. The MOEs for Kern county were approximately one order of magnitude larger than for Fresno county.

MOEs could not be calculated for nBM due to the limited toxicity and air monitoring data; however, a reference exposure level for acute exposure to nBM was estimated to be 250 $\mu g/m^3$ or 67.8 ppb based on the NOAEL from the developmental toxicity study in mice. The highest reported daily average air concentration for nBM (28.6 $\mu g/m^3$ or 7.75 ppb) is well below this reference level, but it is above the odor threshold for nBM in humans (0.01 to 1.0 ppb). Offensive odors can trigger symptoms in humans, such as headache and nausea, through indirect physiologic mechanisms such as innate odor aversion, stress-induced illness or aggravating underlying medical conditions.

The risk for oncogenic effects is the product of its oncogenic potency and the exposure dosage. The estimated oncogenic risk for DEF in ambient air ranged from 3.9 x

10^{-7} to 7.1 x 10^{-7} for the Five Points location in Fresno county and from 7.5 x 10^{-8} to 1.3 x 10^{-7} for the rural communities in Kern county. An oncogenic risk level of less than 10^{-6} is generally considered negligible.

Air concentrations of DEF that are below the reference exposure levels are considered sufficient to protect human health. The reference exposure level is the NOAEL in animals (adjusted for differences in respiratory rate between species) divided by an uncertainty factor of 100 to allow for interspecies and intraspecies variation in susceptibility. The reference exposure level (REL) for both acute and seasonal exposure to DEF was 8.8 µg/m^3 (0.68 ppb) based on the NOAEL for blood ChE inhibition. The REL was 43 µg/m^3 (3.3 ppb) for acute and seasonal exposure based on more overt toxicological effects. The air concentration below which there would be no regulatory concern for oncogenic effects, 42 ng/m^3 (3.3 ppt), corresponds to a negligible oncogenic risk level divided by the oncogenic potency.

II. BACKGROUND

A. Regulatory History

S,S,S-Tributyl phosphorotrithioate (DEF) is an organophosphate chemical whose only registered use has been as a cotton defoliant. n-Butyl mercaptan (nBM) is a degradation product of DEF that is volatile and has a strong skunk-like odor. It is produced both during the manufacture of DEF formulations and after application of DEF in the environment. In 1983, the Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency[1] limited the concentration of nBM in formulations of DEF sold or used in California to less than 0.1% due to public concern about the odor associated with the use of DEF (California Administrative Code, Title 3, Section 6361). Despite the use of low-odor formulations, DPR continues to receive odor-related complaints from residents in cotton-growing regions. Public health concerns were also raised because of evidence that DEF caused delayed neuropathy (see section XI. Neurotoxicity). DPR was given responsibility to identify and control pesticides that are toxic air contaminants (TACs) under Assembly Bill 1807 (AB1807) that was passed in 1983. In 1986, DEF was added to the candidate list of TACs under AB1807. For more information on the regulatory background of DEF, see Part A, Environmental Fate, of the report on DEF as a TAC. The purpose of this health assessment is to evaluate the potential health effects in the general public from exposure to DEF in ambient air due to its use as a cotton defoliant. This health assessment will be the basis for determining whether to list DEF as a TAC.

B. Mechanism of Action

DEF causes defoliation by inducing early leaf abscission through changes in the levels of plant hormones (Ware, 1978). Defoliation occurs 4 to 7 days after treatment. The closely related cotton defoliant, tributyl phosphorotrithioite (merphos), is rapidly converted to DEF by oxidation within a few hours after exposure to air (Obrist and Thornton, 1978).

The toxicity of DEF to animals is due to its inhibition of various esterases, primarily acetylcholinesterase (AChE) and neuropathy target esterase (NTE). AChE is also called specific or true cholinesterase and is found near cholinergic synapses, in some organs (e.g. lung, spleen, gray matter) and in erythrocytes (Lefkowitz *et al.*, 1990). Normally, AChE metabolizes acetylcholine to acetate and choline, which results in the termination of stimulation to dendritic nerve endings and motor endplates. Acetylcholine is the neuro-chemical transmitter at endings of

[1] Prior to 1991, DPR was part of the California Department of Food and Agriculture.

postganglionic parasympathetic nerve fibers, somatic motor nerves to skeletal muscle, preganglionic fibers of both parasympathetic and sympathetic nerves, and certain synapses in the central nervous system (CNS) (Murphy, 1986).

The inhibition of AChE results in the accumulation of endogenous acetylcholine in nerve tissue and effector organs. In acutely toxic episodes, muscarinic, nicotinic and CNS receptors are stimulated with characteristic signs and symptoms occurring throughout the peripheral and central nervous systems (Ellenhorn and Barceloux, 1988; Murphy, 1986). Muscarinic effects can include increased intestinal motility, bronchial constriction and increased bronchial secretions, bladder contraction, miosis, secretory gland stimulation and bradycardia. Nicotinic effects include muscle weakness, twitching, cramps and general fasciculations. Accumulation of acetylcholine in the CNS can cause headache, restlessness, insomnia, anxiety and other non-specific symptoms. Severe poisoning results in slurred speech, tremors, ataxia, convulsions, depression of respiratory and circulatory centers and, eventually, coma.

NTE inhibition in the brain of greater than 70% is associated with the organophosphate-induced delayed neuropathy (OPIDN) produced by some organophosphate compounds with acute exposure (Carrington, 1989; Abou-Donia and Lapadula, 1990; Lotti, 1992). Slightly lower levels of inhibition (~50%) are associated with OPIDN with chronic exposure. The physiological or biochemical role of NTE is unknown at this time. Aging of the phosphorylated enzyme (loss of an alkyl group) apparently is also important in the induction of this neuropathy.

Butyrylcholinesterase (BuChE), sometimes referred to as plasma cholinesterase (ChE), pseudo-cholinesterase, or serum esterase, is also inhibited by DEF. Any reference in this document to "cholinesterase", without specifically indicating that the enzyme is serum or plasma cholinesterase (ChE), should be interpreted as acetylcholinesterase (AChE). BuChE only occurs to a limited extent in neuronal elements of the central and peripheral nervous systems. In addition to plasma, it is also present in the liver, lung and other organs, although its physiological function is unknown (Lefkowitz *et al.*, 1990; Brimijoin, 1992; U.S. EPA, 1993; Pantuck, 1993). An atypical genetic variant of plasma cholinesterase has been associated with an increased susceptibility to various drugs, such as succinylcholine and cocaine (Lockridge, 1990; Pantuck, 1993). However, it is unclear if this increased susceptibility to certain drugs in people with the atypical plasma ChE translates to a possible adverse effect when plasma ChE is inhibited by organophosphates. In an *in vitro* study, it was shown that the atypical and normal plasma ChE was equally sensitive to the organophosphate inhibitors, diisopropylfluorophosphonate (DFP) and tetraethylpyrophosphonate (TEPP), but the atypical plasma ChE was less sensitive than the normal plasma ChE to 14 other drugs, especially succinylcholine and decamethonium (Kalow and

Davis, 1958). In another study, rats that were depleted of plasma AChE by injecting them intravenously with antibodies specific to this enzyme were not more susceptible to paraoxon toxicity than untreated controls based on their performance in a functional observational battery and AChE activity in the brain and diaphragm (Padilla et al., 1992).

DEF also inhibits carboxylesterase, which is involved in the detoxification of various chemicals, including pesticides. In rats and mice, inhibition of carboxylesterase by DEF resulted in the potentiation of several organophosphate pesticides such as malathion that contain a carboxylic ester group (Murphy *et al.*, 1976). However, in mice DEF also markedly potentiated the toxicity of azinphos-methyl which does not contain any carboxylic ester groups (Gaughan *et al.*, 1980). Inhibition of other detoxification enzymes may be involved. Inhibition of mouse liver microsomal esterases is thought to be responsible for the potentiation of permethrin toxicity by DEF (Gaughan *et al.*, 1980). The absorption of phthalate diesters is reduced in rats by DEF apparently due to its inhibition of esterase activity in the intestinal mucosa (White *et al.*, 1980). DEF is also a potent inhibitor of rat liver arylamidase *in vitro* (Satoh and DuBois, 1973).

III. METABOLISM AND PHARMACOKINETICS

A. Introduction

The most extensive study of the metabolism and pharmacokinetics of DEF was conducted in rats by Kao and coworkers (1991). This study was acceptable to DPR based on the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guidelines. Other limited data in rats and other species were available primarily in the area of metabolism. Two studies in laying hens and lactating goats were conducted in accordance with FIFRA guidelines for residue studies (Hall, 1991; Sahali, 1991). The remaining studies were published reports and, as such, may not meet FIFRA guidelines.

B. Absorption

[^{14}C] DEF was administered to 5 rats/sex/dose in a single dose by oral gavage at 5 or 100 mg/kg or in 14 consecutive doses at 5 mg/kg/day (Kao *et al.*, 1991). Approximately 90-100% of the total dose was excreted in the urine and feces within 72 hours after dosing. Most of the radioactivity was excreted within 24 hours after a single dose at 5 mg/kg (M: 91%; F :87%) or 100 mg/kg (M: 75%; F: 57%). A similar percentage (M: 89%; F: 85%) was excreted within 24 hours after 14 consecutive doses at 5 mg/kg/day. The majority of the radioactivity was excreted in the urine after a single dose at 5 mg/kg (M: 55%; F: 66%) or 100 mg/kg (M: 60%;F: 70%). A slightly higher percentage (M: 73%; F: 80%) was excreted in the urine after 14 consecutive doses at 5 mg/kg/day.

Male rats had [^{14}C] DEF (98.9% - mixed in distilled water with DEF 6 blank formulation) applied to their shaved backs at 1.93, 12.4 and 100 µg/cm^2 for 10 hours (Schroeder, 1992). The application site was protected by a non-occlusive cover of a Teflon-laminated filter and a carbon-impregnated material. Four rats/dose were sacrificed at 1, 4, 10 and 168 hours (7 days). The amount excreted in the urine over 7 days ranged from 25.8 to 36.0% of the applied dose decreasing from the low to high dose level. On the other hand, the amount excreted in the feces was fairly similar (3.2 to 3.6% of applied dose) at the different dose levels. After correcting for recoveries, the dermal absorption rates were 47.5, 47.9 and 33.9% at 1.93, 12.4, and 100 µg/cm^2, respectively.

The pharmacokinetics of DEF after intravenous administration was not studied due to the low water solubility of DEF. However, based on the nearly complete elimination of DEF by the urinary route when applied dermally to rats (Schroeder, 1992), it was assumed the amount

excreted by the biliary route is insignificant when DEF is administered orally. Based on this finding, DPR assumed that with oral administration most of the radioactivity in the feces was unabsorbed material. Therefore, the oral absorption rate for DEF was estimated to be 70% based on the approximate average urinary excretion for all dosing regimens.

There were no data available on the absorption of DEF by the inhalation route.

C. Distribution

In laying hens administered DEF at 50 mg/kg by the oral and dermal routes, the half-lives were 2.7 and 3.8 days, respectively, based on the plasma concentration curve (Abou-Donia *et al.*, 1984). Hall (1991) measured residues in the liver, fat, muscle, and eggs of laying hens given [^{14}C] and [^{35}S] DEF at 4 mg/kg/day in gelatin capsules for 3 consecutive days. Four hours after the last dose, the highest residues were found in liver followed by internal eggs, muscle, and fat.

Tissue residues were also determined in lactating goats 21 hours after receiving [^{14}C] DEF at 0.82 or 0.85 mg/kg/day in gelatin capsules for 3 consecutive days (Sahali, 1991). Of the four tissues examined (muscle, fat, kidney, liver), the liver had the highest residues and the muscle had the lowest. The residues in milk were between those in fat and muscle.

The most extensive residue analysis of tissues was conducted by Kao *et al.* (1991) in rats administered [^{14}C] DEF by oral gavage at 5 or 100 mg/kg. Less than 3% of the total dose was found in the tissues and carcasses 72 hours after dosing. The highest residue levels were found in the liver, followed by fat, lung, kidney, blood, gastrointestinal tract, spleen, bone, heart, gonads, muscle, and brain.

D. Biotransformation

One of the initial steps in the metabolism of DEF appears to be its oxidation to an active metabolite, such as a sulfoxide. A more potent ChE inhibitor was formed with the addition of microsomal fractions or purified cytochrome P-450 isozymes from mouse liver to DEF *in vitro* (Wing *et al.*, 1984; Levi and Hodgson, 1985). The activity of cytochrome P-450 and aniline hydroxylase were induced in a dose-related manner in hens administered DEF at 100, 200, 500 or 1,000 mg/kg by either the dermal or oral route with the induction being greater by the dermal route (Lapadula *et al.*, 1984). However, a significant increase in cytochrome P-450 activity was not found when mice were administered DEF intraperitoneally once at 31 mg/kg or orally at 100 mg/kg/day for 3 days (Skrinjaric-Spoljar *et al.*, 1971; Fabacher *et al.*, 1980). Liver O-

demethylase also was not induced in the mouse study conducted by Fabacher *et al.* (1980), but it was induced in hens administered DEF orally and dermally at 3.5 and 7 mg/kg/day, respectively, for 90-100 days (Hansen *et al.*, 1982). DEF is probably further metabolized by conjugation with glutathione based on the slight induction of glutathione S-transferases after oral administration to mice at 100 mg/kg/day for 3 days (Kulkarni *et al.*, 1980).

Hall (1991) and Sahali (1991) were unable to identify any of the metabolites in the tissues analyzed. Based on the complexity of the metabolite profiles and the extreme polarity of many of the metabolites in these tissues, these investigators suggested that most of the parent compound had been incorporated into natural constituents. Radioactive residues were detected in fatty acids and proteins in goat tissues (Sahali, 1991).

Kao *et al.* (1991) proposed a metabolic pathway for DEF, which involves the initial hydrolysis of DEF to S,S-dibutyl phosphorodithioate and nBM (Figure 1). nBM is converted to the fatty acid, butyric acid, which may be further metabolized through the usual metabolic pathways for fatty acids. S,S-Dibutyl phosphorodithioate is metabolized to nBM and phosphate. Hur and coworkers (1992) proposed a similar metabolic pathway based on the isolation of two metabolites (S,S-dibutyl phosphorodithioate and S,S-dibutyl phosphorothioic acid) after incubation of DEF with mouse liver microsomes and in rat urine after intraperitoneal injection of DEF at 100 mg/kg. They proposed that these metabolites were formed via DEF sulfoxide and S,S-dibutyl,S-1-hydroxybutyl phosphorotrithioate, respectively, which are reactive intermediates formed by microsomal mixed function oxidases (MFOs), such as cytochrome P-450.

Abou-Donia and coworkers isolated nBM in the plasma and excreta of hens administered a single oral dose of DEF at 400 mg/kg or 30 daily oral doses at 20-80 mg/kg/day (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators concluded that a portion of orally administered DEF is converted to nBM in the gastrointestinal tract through hydrolysis. These studies are discussed in more detail in the Neurotoxicity section of the Toxicology Profile.

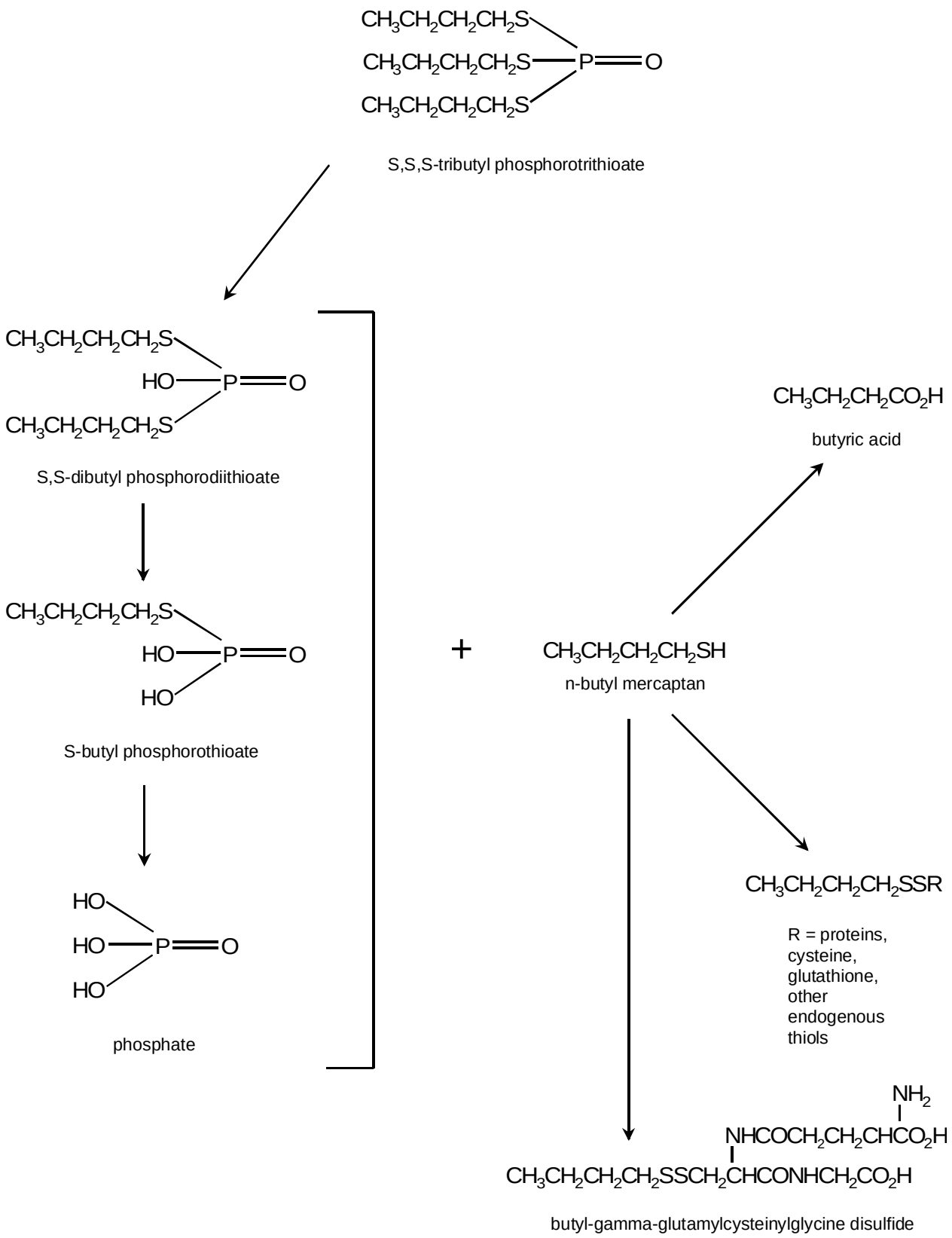

Figure 1. Proposed Metabolic Pathway for DEF (Kao *et al.*, 1991)

E. Excretion

As mentioned previously under absorption, the major route of excretion in rats was the urinary route with an average excretion rate at 72 hours between 55 to 80% (*Kao et al.*, 1991). In the first 24 hours, the percent excreted in the urine was affected by both dosage (decreased with increasing dosage) and sex (lower in males). The average amount excreted in the urine at 24 hours was lowest (M: 44%; F: 40%) in rats administered a single dose at 100 mg/kg; however, by 72 hours the total amount excreted in the urine was similar (M: 60%; F: 70%) to rats administered a single dose at 5 mg/kg (M: 55%; F: 66%). The urinary excretion was highest after administration of DEF at 5 mg/kg/day for 14 consecutive days at both 24 hrs (M: 67%; F: 72%) and 72 hrs (M: 73%; F: 80%), suggesting more efficient absorption and/or metabolism with continued exposure. The average urinary excretion was higher in females on all of the dosing regimens at 72 hours (M: 55-73%; F: 66-80%), suggesting the absorption and/or metabolism of DEF is more efficient in females than males. A significant amount of DEF was also eliminated in the feces of rats within 72 hours following a single oral dose of DEF at 5 mg/kg (M: 42%; F: 30%) or 100 mg/kg (M: 38%; F: 27%). The fecal excretion was slightly lower after 14 consecutive doses at 5 mg/kg/day (M: 24%; F: 15%). Only 1% was excreted as CO_2 in expired air for either sex.

F. Conclusions

DEF appears to be readily absorbed by the oral route and rapidly metabolized in the species examined. The oral absorption rate was assumed to be 70% based on the average urinary excretion in rats from all dosing regimens.

IV. ACUTE TOXICITY

A. Introduction

Illness reports describing acute toxic effects of DEF in humans have been described in detail in the Exposure Assessment Document for DEF as a Toxic Air Contaminant (TAC) and are only briefly summarized here. The adverse effects observed in laboratory animals with the standard battery of acute toxicity tests (oral and dermal LD_{50} tests, inhalation LC_{50} test, dermal and ocular irritation tests, and dermal sensitization test) are described in more detail. These tests were available for both the technical grade DEF and the formulations, which contain approximately 70% DEF. Traditionally, DPR toxicologists have used the term "no-observed-effect level" or " NOEL" to identify dose levels in animal or human studies at which no adverse effects have been seen. After reviewing the Health Assessment document for the Evaluation of DEF as a TAC, the Scientific Review Panel (SRP) for TACs requested that the term no-observed-adverse-effect level (NOAEL) be used instead. Consequently, dose levels that may have been identified as NOELs in other documents for DEF have been identified as NOAELs in this document. Nine of the 17 available acute toxicity tests for DEF were acceptable based on FIFRA guidelines. Several acute toxicity tests were also conducted on the degradation product, nBM, and the metabolite, 3-hydroxybutyl-methyl sulfone; however, none of these tests met FIFRA guidelines. Acute effects observed in subchronic, developmental, and neurotoxicity studies are not included here, but are discussed later under those sections. All acute effects are summarized under Acute Toxicity in the Hazard Identification section.

B. Human Health Effects

In 1977, the California Department of Food and Agriculture (CDFA) published a report summarizing several hundred complaints it had received that were associated with DEF (Maddy and Peoples, 1977). The complaints usually involved wheezing, coughing, nausea, and other discomforts that were attributed to the degradation product, nBM, a volatile degradation product of DEF with a strong skunk-like odor. This odor apparently can be detected by humans at air concentrations as low as 0.01 ppb (Santodonato *et al.*, 1985).

Approximately 13 drums containing DEF and Merphos (S,S,S-tributyl phosphorotrithioite) were damaged on a ship in transit from Mexico to Australia (McLeod, 1975). Caustic soda was used in the process of cleaning up the damaged drums in Auckland, New Zealand, which resulted in increased liberation of nBM. Over 600 people were seen at a local hospital with various complaints. Symptoms observed in 49 cases were attributed to organophosphate poisoning

(excessive salivation, sweating, muscle weakness, fatigue, nausea, vomiting, diarrhea, and miosis); however, no cholinesterase inhibition was found. Symptoms attributed to nBM (headache, dizziness, dry mouth and throat constriction) were reported in another 192 cases. It was estimated that the air levels of nBM exceeded 0.5 ppm (ACGIH TLV-TWA) and in some places exceeded 10 ppm. It was not possible to categorize the symptoms observed in the remaining cases. It was reported that panic may have been a factor in some of these cases since there had been widespread coverage of the spill in the news media.

More recently, the California Department of Health Services did an epidemiology study in which they examined the relationship of the health symptoms and community exposure to cotton defoliants (Scarborough *et al.*, 1989). Four-hundred and six residents in six agricultural communities in the San Joaquin Valley were surveyed by phone during the time of cotton defoliation. They found a significantly greater risk for eye and throat irritation, rhinitis, fatigue, shortness of breath, nausea and diarrhea in the high exposure group (people who lived or worked within one mile of a cotton field that had been treated within the previous two weeks). In the high-exposure group, there was also a significantly greater risk for these self-reported symptoms in the subgroup noticing a strong odor, suggesting that DEF or nBM was the causative agent.

California Health and Safety Code requires that any illness suspected of being caused by a pesticide be reported by the examining physician to the county health officer within 24 hours (CCR, Title 17, Section 2950). There were a total of 16 illness and injury cases associated with exposure to DEF and DEF in combination with other pesticides in California from 1982 through 1991 (DPR, 1994). These cases were mostly due to occupational exposure (12 occupational and 4 non-occupational cases) and resulted from close contact with DEF products. Of the 16 cases, 11 were systemic illnesses, two were eye injuries, and three were respiratory illnesses. Systemic poisoning due to exposure to DEF and DEF in combination with other pesticides was positively identified (definite) in four cases. ChE depression was observed in three of the four definite systemic cases. One systemic case was classified as probable and six others as possible cause of the acute poisoning.

There was also a published case report about a 28-year-old agricultural worker who developed symptoms similar to delayed neuropathy after he spilled merphos on his arm (Fisher, 1977). The worker did not develop any acute symptoms, but four days later his hands and arms became weak. He finally sought medical attention six days after exposure when he could barely move his arms or legs. After admission to the hospital, his plasma cholinesterase level was normal despite his symptoms. Eight days later, complete facial paralysis developed. Electromyography demonstrated decreased voltage of action potentials, delayed conduction velocity, increased

insertional activity, and denervation potentials. The worker completely recovered after fourteen weeks of intensive physical therapy.

C. Animal Studies

1. Technical Grade DEF

The acute toxicity of technical grade DEF in animals is summarized in Table 1. In an acceptable acute inhalation study with technical grade DEF, the lowest-observed-adverse-effect levels (LOAELs) were 2,920 and 1,590 mg/m^3 (467 and 254 mg/kg)[2] for male and female rats, respectively with a 4-hour, nose-only exposure (Warren, 1990). Analysis of particle size indicated that the mass mean aerodynamic diameter was approximately 1.5 µm and that greater than 87% of the particles were less than 2 µm. Based on the particle size analysis, DPR toxicologists assumed that 100% of DEF in the inhaled air reached the alveoli and was absorbed. Unthriftiness (M: 6/6, F: 6/6), hypoactivity (M: 3/6, F: 4/6), urine stains (M: 4/6, F: 3/6), nasal discharge (M: 3/6, F: 3/6), red eye discharge (M: 3/6, F: 2/6), lacrimation (M: 0/6, F: 3/6), ataxia (M: 2/6, F: 1/6), tremors (M: 0/6, F: 2/6), death (M: 1/6, F: 1/6), excitability (M: 0/6, F: 1/6), vocalization (M: 0/6, F: 1/6), dyspnea (M: 0/6, F: 1/6), red turbinates (M: 1/6, F: 0/6), and firm zones in the lungs (M: 1/6, F: 0/6) were observed at the LOAEL. A no-observed-adverse-effect level (NOAEL) was not established in this study. In another 4-hour (nose only) acute inhalation study in rats, a NOAEL was established at 77 mg/m^3 (12.3 mg/kg)[2] based on abnormal behavior, including decreased preening and lethargy in animals at 130 mg/m^3 (20.8 mg/kg) and higher (Thyssen, 1978a). However, this study had several deficiencies including no summary of clinical signs by dose group, no gross necropsy, and no analysis of particle size.

In an acceptable acute oral toxicity study, death occurred at 192 and 294 mg/kg and higher in female and male rats, respectively (Sheets, 1991a). The NOAEL was less than 192 mg/kg for females and 294 mg/kg for males based on urine stain (M: 3/5, F: 4/5), red lacrimal stain (M: 4/5, F: 2/5), clear lacrimation (M: 3/5, F: 3/5), diarrhea (M: 0/5, F: 5/5), perianal stain

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[2] Dose was estimated from air concentration in mg/m^3 using Equation 1 in Appendix A. The respiratory rate for a rat was assumed to be 0.16 m^3/kg/4 hrs (Zielhuis and van der Kreek, 1979).

Table 1. The Acute Toxicity of Technical Grade DEF (95-99.7%)

Species	Sex	Results	References[a]
		Inhalation LC_{50}	
Rat	M	4,000 mg/m^3 (4-hr, nose only)	1
	F	1,600 mg/m^3 (4-hr, nose only)	
	M	4,650 mg/m^3 (4-hr, nose only)	2*
	F	2,460 mg/m^3 (4-hr, nose only)	
		Acute Oral LD_{50}	
Rat	M	435 mg/kg	3*
	F	234 mg/kg	
		Acute Dermal LD_{50}	
Rabbit	M/F	1,093 mg/kg	4*
		Primary Dermal Irritation	
Rabbit	M/F	Mild Irritant	5,6*
		Primary Eye Irritation	
Rabbit	M/F	Mild Irritant	5,7*
		Dermal Sensitization	
Guinea Pig	M/F	Non-Sensitizer	8

a References: 1. Thyssen, 1978a; 2. Warren, 1990; 3. Sheets, 1991a; 4. Sheets and Phillips, 1991; 5. Crawford and Anderson, 1972a; 6. Sheets and Fuss, 1991; 7. Sheets and Phillips, 1992a; 8. Sheets, 1990.

* Acceptable study based on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines

(M: 1/5, F: 4/5), red nasal stain (M: 4/5, F: 0/5), decreased activity (M: 2/5, F: 0/5), salivation (M: 1/5, F: 0/5), dyspnea (M: 1/5, F: 0/5), wheezing (M: 1/5, F: 0/5), and clear nasal stain (M: 1/5, F: 0/5).

After dermal administration, deaths were observed at 1,000 mg/kg and higher in rabbits (Sheets and Phillips, 1991). The NOAEL was less than 500 mg/kg based on tremors (1/10), muscle fasciculations (10/10), erythema at the site of application (9/10), hypoactivity (2/10), clear

nasal discharge (2/10), white nasal discharge (1/10), ataxia (1/10), increased reactivity (1/10), clear lacrimation (1/10), and clear lacrimal stain (1/10). This study was also acceptable based on FIFRA guidelines. Technical grade DEF was only mildly irritating to the skin and eyes of rabbits (Crawford and Anderson, 1972a; Sheets and Fuss, 1991; Sheets and Phillips, 1992a) and did not induce a sensitization response in guinea pigs using the Buehler patch test (Sheets, 1990).

In environmental temperatures below 30°C, hypothermia has been observed in rats, mice, and guinea pigs, but not rabbits after a single dose of DEF between 20 and 200 mg/kg by the oral, intraperitoneal or intravenous route (Ray, 1980; Ray and Cunningham, 1985). At doses greater than 100 mg/kg, the hypothermia persisted for several days. The hypothermia was associated with piloerection, sluggishness, and irritability, but a high degree of motor control even when body temperature reached 30°C. As body temperatures dropped below 30°C, deaths occurred usually after prolonged hypothermia. The hypothermia appears to be due to a block of shivering and non-shivering thermogenesis with little effect on basal metabolism, heat conservation or motor control. The investigators suggested a selective action on a central thermogenic control process may be involved. Other research indicates that the hypothermia associated with organophosphates is due to central AChE inhibition because it is antagonized by centrally active antiChE drugs, such as atropine, but not by peripherally active antiChE drugs, such as 2-PAM (Kenley *et al.*, 1982). A NOAEL could not be established for this effect from these studies, although the effect was minimal with intraperitoneal injection of DEF at 20 mg/kg.

2. DEF Emulsifiable Concentrates

The acute toxicity of DEF emulsifiable concentrates in animals is summarized in Table 2. The signs observed after treatment with DEF emulsifiable concentrates were similar to those observed with technical DEF. With inhalation exposure in rats, the LOAEL was 540 mg formulation/m^3 (4-hr, nose-only) (Warren and Tran, 1992). Hypoactivity, lacrimation, red nasal discharge, and unkempt appearance were observed at this dose, but no mortalities or gross lesions. Red lungs and nasal turbinates were observed at necropsy at higher doses. A NOAEL was not established for this study. The acute toxicity studies for the formulation were not used in this risk assessment because the general public is not exposed to it in the same form for two reasons. First, the formulation is diluted at least 16- fold in water before applying. Second, the main inert ingredient in the formulation is a solvent which has probably dissipated by the time DEF reaches the general public.

Table 2. The Acute Toxicity of DEF Emulsifiable Concentrates (70%)

Species	Sex	Results	References[a]
		Acute Inhalation LC_{50}	
Rat	M	> 1,350 mg/m^3 (1-hr)	1
	F	> 1,450 mg/m^3	
	M	3,550 mg/m^3 (4-hr, nose only)	2*
	F	2,340 mg/m^3	
Mice	M	2,120 mg/m^3 (30-min)	3
		Acute Oral LD_{50}	
Rat	M	570-712 mg/kg	4,5*
	F	349 mg/kg	
		Acute Dermal LD_{50}	
Rabbit	M/F	300 mg/kg	6
Rat	M/F	> 2,000 mg/kg	7*
		Primary Dermal Irritation	
Rabbit	M/F	Corrosive	8,9*
		Primary Eye Irritation	
Rabbit	M/F	Severe Irritant	8

a References: 1. Kimmerle, 1972; 2. Warren and Tran, 1992; 3. DuBois and Meskauskas, 1968; 4. Crawford and Anderson, 1972b; 5. Sheets and Phillips, 1992b; 6. Crawford and Anderson, 1972c; 7. Astroff and Phillips, 1992a; 8. Crawford, 1971; 9. Sheets and Phillips, 1992c.

* Acceptable study based on the FIFRA guidelines.

The lowest LOAEL by the oral route was 290 mg formulation/kg in female rats (Sheets and Phillips, 1992b). The effects reported at the LOAEL were tremors, hypoactivity, increased reactivity, vocalizations, hunched back, labored breathing, muscle fasciculations, lacrimation, nasal, ocular, oral and perianal stains. Compound-related gross lesions in animals that died included discolored stomach zones, red fluid in the bladder, gas/fluid in the intestines, and fluid in the abdomen.

There appeared to be a species difference in susceptibility based on the dermal LD_{50} values for rabbits and rats (300 vs. >2,000 mg formulation/kg, respectively); however, the NOAELs

appear to be similar. With rabbits, a NOAEL was established at 106 mg formulation/kg based on unspecified cholinergic signs at 212 mg formulation/kg (Crawford and Anderson, 1972c). In rats, a NOAEL was not established; however, the LOAEL was 500 mg formulation/kg in both sexes of rats based on ataxia, increased reactivity, irritation at application site, and nasal and perianal stains (Astroff and Phillips, 1992a). No mortalities or gross lesions were seen at the LOAEL. A DEF emulsifiable concentrate produced severe skin and eye irritation in rabbits (Crawford, 1971; Sheets and Phillips, 1992c) which appeared to be due primarily to the inert ingredients since the technical grade DEF was only mildly irritating (Crawford and Anderson, 1972a). No studies were available on the dermal sensitization potential of the DEF emulsifiable concentrates.

3. N-Butyl Mercaptan

The acute toxicity of nBM, a major degradation product of DEF, was examined by one laboratory (Table 3) (Fairchild and Stokinger, 1958). Based on the LD_{50} and LC_{50} values, nBM appears to be less lethal than DEF. The inhalation LC_{50} estimates for nBM ranged from 2,500-4,020 ppm (9,202-14,798 mg/m^3) which were significantly higher than those for DEF which ranged from 1,600-4,650 mg/m^3. The oral LD_{50} estimate for nBM (1,500 mg/kg) was also significantly higher than those for DEF (234-435 mg/kg). The clinical signs observed after exposure to nBM were indicative of CNS depression. The signs observed in approximate order of appearance included restlessness, increased respiration, incoordination, muscular weakness, skeletal muscle paralysis, cyanosis, lethargy, sedation, respiratory depression, coma, and death. The signs were similar regardless of route of exposure, except that with oral exposure, where diarrhea was also observed and with inhalation exposure, where watery eyes and sneezing were also observed. nBM also produced slight irritation in an ocular irritation test with rabbits. The pathological findings with all routes of exposure included indications of kidney damage (cloudy swelling of the tubules and hyaline casts in the lumina) and liver damage (lymphocytic infiltration and necrotic foci with small hemorrhages). With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lung were also observed. The systemic and local toxicity after exposure to nBM by the dermal route was not examined. The lungs appear to be an important route of excretion for nBM because a strong odor was detected in the expired air of animals regardless of the route of exposure. It was not possible to establish a NOAEL by any route since the incidences of clinical signs and pathological lesions were not summarized by dose level.

Table 3. The Acute Toxicity of Technical Grade n-Butyl Mercaptan

Species	Sex	Results	Reference[a]
		Acute Inhalation LC_{50}	
Rat	M	4,020 ppm (4-hr)	1
Mice	M	2,500 ppm	
		Acute Oral LD_{50}	
Rat	M	1,500 mg/kg	1
		Acute Intraperitoneal LD_{50}	
Rat	M	399 mg/kg	1
		Primary Eye Irritation	
Rabbit	M	Slight Irritant	1

a Reference: 1. Fairchild and Stokinger, 1958.

4. 3-Hydroxybutylmethyl Sulfone Metabolite

In an acute oral toxicity study, 5 female Sprague-Dawley rats were given 3-hydroxy-butylmethyl sulfone in water by gavage at 0 or 2,000 mg/kg (Astroff and Phillips, 1992b). There were no mortalities, reduction in body weights or treatment-related gross lesions. Ataxia, lacrimation, hypoactivity, hyperactivity, increased reactivity, and hunched back were observed in the treated animals. The LD_{50} was greater than 2,000 mg/kg. A NOAEL could not be established for this study. The toxicological significance of this metabolite is uncertain at this time, but it appears to be less toxic than DEF. This study had several deficiencies including only females tested and no analysis of dosing material.

D. Conclusions

The acute effects from exposure to DEF in humans included cholinergic signs such as miosis, excessive salivation and sweating. However, investigators attributed many of the symptoms, such as eye and throat irritation, coughing, and difficulty in breathing, to the degradation product, nBM. Nausea, vomiting and diarrhea were also common symptoms, but it is

unclear if these were due to DEF or nBM. An acute NOAEL could not be established from the available human data due to the lack of accurate exposure data. Clinical signs observed in animals exposed to DEF were typical cholinergic signs (e.g., ataxia, tremors, facial and urogenital stains). With inhalation exposure, dyspnea, red turbinates, and firm zones in the lungs were also reported. Erythema was also observed with dermal exposure. The effects observed in animals exposed to nBM were typical of CNS depression. The pathological findings included kidney and liver damage with all routes of exposure and lung damage with inhalation exposure. Ocular irritation was also observed. When comparing LD_{50}/LC_{50} values for the different routes in animals, technical grade DEF appears to be slightly more lethal by the inhalation route than the oral route and least lethal by the dermal route. The higher dermal LD_{50} values also suggest that absorption of DEF by the dermal route is slower or incomplete. The LC_{50}/LD_{50} values suggest that nBM is less acutely lethal than DEF. A NOAEL was not established in an acceptable inhalation LC_{50} study for DEF where death, cholinergic signs, red turbinates and "firm zones" were observed in rats at the lowest dose tested, 1,590 mg/m^3 (254 mg/kg). A NOAEL was observed at 77 mg/m^3 (12.3 mg/kg) in unacceptable inhalation LC_{50} study for DEF in which decreased preening and lethargy were observed in rats at 130 mg/m^3 (20.8 mg/kg) and higher. NOAELs were not established in any other acute toxicity studies with technical grade DEF or nBM.

V. SUBCHRONIC TOXICITY

A. Introduction

Seven subchronic studies of variable exposure duration were available for DEF. Three were inhalation studies with rats. The exposure period was only 2-3 weeks in two of the inhalation studies, but was 13 weeks for a third study. Three oral studies were conducted using different species (mice, rats, and dogs) with exposure periods ranging from 8 to 13 weeks. A 3-week dermal toxicity study was also conducted using rabbits. Only the 13-week inhalation study and the 3-week dermal study met the FIFRA guidelines. Other subchronic effects are described under the Reproductive and Developmental Toxicity sections and are summarized under Subchronic Toxicity in the Hazard Identification section.

B. Inhalation Studies

Ten Wistar-II rats/sex/group were exposed (nose only) to DEF (95%) at analytical air concentrations of 0, 2, 7 or 32 mg/m^3 (0, 0.5, 1.7 or 7.7 mg/kg/day)[3] for 6 hour/day, 5 days/week for 3 weeks (Thyssen, 1978b). Animals exposed to 32 mg/m^3 exhibited slight behavioral abnormalities, including lethargy and decreased preening. The high-dose animals also had increased absolute and relative adrenal gland and spleen (females only) weights and slight inflammatory lung alterations at necropsy. The increased organ weights were not considered toxicologically significant by DPR toxicologists since there were no apparent treatment-related histological changes in the adrenal gland and spleen. There was a significant reduction in the mean plasma ChE activity at 7 mg/m^3 (M: 64%; F: 52% of controls) and 32 mg/m^3 (M: 36%; F: 15% of controls) at study termination. The mean erythrocyte ChE activity was reduced at 32 mg/m^3 (M: 76%; F: 73% of controls). The mean brain ChE activity was also reduced at 32 mg/m^3 (F: 73% of controls). No effects on body weights, hematology, clinical chemistry, urinalyses or gross pathology were found. The NOAEL was 7 mg/m^3 (1.7 mg/kg/day) based on the brain ChE inhibition, clinical signs, and histological changes in the lung. This study had major deficiencies including inadequate exposure duration (< 90 days), inadequate hematology, clinical chemistry, and histopathology, and no analyses of airflow, particle size or temperature in the chambers during exposure.

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[3] Dose was estimated from air concentration in mg/m^3 using Equation 1 in Appendix A. The respiratory rate for a rat was assumed to be 0.24 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979). A 100% respiratory retention and absorption was assumed.

Ten Bor:WISW (SPF-Cpb) rats/sex/dose were exposed (nose only) to DEF (98%) at analytical air concentrations of 0, 0.27, 2.6, 13.3 or 62.5 mg/m^3 (0, 0.06, 0.6, 3.2 or 15 mg/kg/day)^3 for 6 hrs/day, 5 days/wk in a two-week range-finding study (Pauluhn, 1991). Particle size analysis indicated that greater than 99% of the particles were less than 3 µm. Therefore, DPR assumed that 100% of the DEF in inhaled air reached the alveoli and was absorbed. At 62.5 mg/m^3, rats exhibited hypoactivity, aggressive behavior, vocalization, piloerection, exophthalmos, bradypnea, dyspnea, and slight hypothermia. Reduced activity, bradypnea, and vocalization (females only) were observed in at least 5 of 10 animals by day 3 of the study at 62.5 mg/m^3. At 2.6 mg/m^3 and higher, some females displayed a more pronounced tail-pinch response on day 7. The toxicological significance of this effect is unknown. The mean plasma ChE activity was reduced at 62.5 mg/m^3 (M: 42%; F: 13% of controls) at the study termination. The mean erythrocyte ChE activity was reduced at 13.3 mg/m^3 (M: 62% of controls) and 62.5 mg/m^3 (M: 27%; F: 25% of controls). Significantly reduced mean brain ChE activity (61% of control activity) was seen in females at 62.5 mg/m^3. In males, a significant reduction in relative liver weights were observed at 13.3 and 62.5 mg/m^3. In females, a significant reduction in absolute spleen weights were observed at 62.5 mg/m^3. The reduction in organ weights was not considered toxicologically significant since there were no treatment-related histological changes in these organs. The acute NOAEL was 13.3 mg/m^3 (3.2 mg/kg) based on the reduced activity, bradypnea, and vocalization by day 3. The subchronic NOAEL was also 13.3 mg/m^3 (3.2 mg/kg) based on the clinical signs and brain ChE inhibition (F: 61% of controls). This study was designed as a range-finding study; therefore, it did not meet the FIFRA guidelines for a subchronic study because the exposure period was short and there was no hematology, clinical chemistry or histopathology examination.

In a 13-week subchronic inhalation toxicity study, 10 Bor:WISW (SPF-Cpb) rats/sex/dose were exposed (nose only) to analytical air concentrations of DEF (98%) at 0, 0.9, 2.4, 12.2 or 59.5 mg/m^3 (0, 0.2, 0.6, 2.9 or 14.3 mg/kg/day)^3 for 6 hours/day, 5 days/week (Pauluhn, 1992). The particle size analysis indicated that greater than 99% of the particles were less than 3 µm. Therefore, DPR assumed that 100% of the DEF in inhaled air reached the alveoli and was absorbed. Various clinical signs were observed in animals at 59.5 mg/m^3 including reduced motility, bradypnea, irregular breathing, dyspnea, increased aggressiveness, miosis, exophthalmos, vocalization, piloerection, ungroomed coat, convulsions, blepharospasm (spasm in the eyelid muscle resulting in more or less complete closure of the eyelid), and hypothermia (females only). Some of these signs were observed within the first few days of dosing and, therefore, were considered acute effects (Table 4). Most of these signs do not appear to be cholinergic in origin, but may reflect a localized response in the lungs. However, they appear to

Table 4. Incidence of Clinical Signs in Rats During the First 3 Days of Exposure to DEF in a 90-Day Inhalation Study

	Dose Level (mg/m^3)									
	0		0.9		2.4		12.2		59.5	
	M	F	M	F	M	F	M	F	M	F
Reduced motility	0[a]	0	0	0	0	0	0	0	10(2)	9(1)
Bradypnea	0	0	0	0	0	0	0	0	8(2)	10(2)
Piloerection	0	0	0	0	0	0	0	0	5(3)	10(3)
Ungroomed coat	0	0	0	0	0	0	0	0	1(2)	8(1)
Vocalization	0	0	0	0	0	0	0	0	1(3)	7(1)
Irregular breathing	0	0	0	0	0	0	0	0	1(2)	2(1)
Inc. startle response	0	0	0	0	0	0	0	0	0	1(2)

[a] The incidence per group (10 rats/sex/dose) during the first 3 days of exposure. The number in parentheses is the day of the study on which this sign was first observed.

be treatment-related since none of these were observed in the lower treatment groups or the controls. There was no treatment-related effect on reflexes, body weight, or clinical chemistry. The mean erythrocyte count, hematocrit, and hemoglobin values was reduced significantly in males at 59.5 mg/m^3 (8%, 7%, and 8%, respectively). The mean erythrocyte count, hematocrit and hemoglobin values were also reduced in females (3%, 7%, and 6%, respectively), but these differences were not statistically significant. At study termination, the mean plasma ChE activity was reduced at 12.2 mg/m^3 (F: 60% of controls) and 59.5 mg/m^3 (M: 51%; F: 33% of controls). The mean erythrocyte ChE activity was also reduced at 12.2 mg/m^3 (M: 35%; F: 36% of controls) and 59.5 mg/m^3 (M: 19%; F: 13% of controls). The mean brain ChE activity was significantly reduced at 59.5 mg/m^3 only (M&F: 60% of controls). Pale or mottled retinal fundus was noted in females at 59.5 mg/m^3 with the ophthalmological examination. Animals at 59.5 mg/m^3 had evidence of impaired retinal function based on reduced a and b waves in the electroretinographic (ERG) examination; however, histological examination of the eye revealed no evidence of retinal degeneration. Fine fatty droplets in the adrenal cortex and elevated absolute and relative adrenal gland weight were also seen in rats at 59.5 mg/m^3. The acute NOAEL for this study was 12.2 mg/m^3 (2.9 mg/kg/day) based on the reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing and increased startle response. The subchronic NOAEL was also

12.2 mg/m^3 (2.9 mg/kg/day) based on the clinical signs, hematological changes, brain ChE inhibition (M&F: 60% of controls), impaired retinal function, pale retinal fundus, fatty droplets in the adrenal gland, and increased adrenal weights. This study was found acceptable by DPR toxicologists based on FIFRA guidelines.

C. Oral Studies

In a pilot study, 15 CD-1 mice/sex/group were fed DEF (97.7%) in the diet at 0, 10, 30, 90 or 270 ppm (M: 0, 3.4, 9.4, 40 or 140 mg/kg/day; F: 0, 5.6, 14.3, 54 or 132 mg/kg/day) for 8 weeks (Hayes, 1985). No clinical signs or deaths were observed. The mean food consumption was significantly higher in the males at 90 ppm (33%) and 270 ppm (51%) and in females at 90 ppm (29%). There was no effect on body weight gain. The mean plasma ChE activity was markedly reduced at 10 ppm (M: 36%; F: 29% of controls), 30 ppm (M: 13%; F: 8% of controls), 90 ppm (M: 7%; F: 4% of controls), and 270 ppm (M: 5%; F: 4% of controls). The mean erythrocyte ChE activity was reduced at 30 ppm (M: 63%; F: 56% of controls), 90 ppm (M&F: 36%), and 270 ppm (M: 27%; F: 31% of controls). The mean brain ChE activity was only reduced at 270 ppm (M: 74%; F: 71% of control activity). The NOAEL was 90 ppm (M: 40 mg/kg/day; F: 54 mg/kg/day) based on the brain ChE inhibition. This study was designed as a pilot study for an oncogenicity study and, therefore, the exposure period was short. In addition, there was no histopathological examination, no clinical chemistry or hematology, and no analysis of the diet.

Groups of male and female Sprague-Dawley rats were fed diets containing DEF at 0, 5, 10, 20, 50 or 100 ppm (0, 0.25, 0.5, 1.0, 2.5 or 5.0 mg/kg/day)[4] for 3 months (Root and Doull, 1966). A NOAEL of 5 ppm (0.25 mg/kg/day) was reported, but the toxic effects were not indicated. This study had major deficiencies including no summary of the incidence of clinical signs, body weights, food consumption, pathology findings and no clinical chemistry or hematology.

DEF was also administered to groups of male and female beagle dogs in the feed at 0, 5, 10, 20, 50 or 100 ppm (0, 0.125, 0.25, 0.5, 1.25 or 2.5 mg/kg/day)[5] for 3 months (Root and

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[4] Estimated assuming that for a rat 1 ppm in the diet is equivalent to 0.05 mg/kg/day (FDA, 1959).

[5] Estimated assuming that for a dog 1 ppm in the diet is equivalent to 0.025 mg/kg/day (FDA, 1959).

Doull, 1966). Again, a NOAEL of 5 ppm (0.125 mg/kg/day) was reported, but the toxic effects were not indicated. This study also had major deficiencies including no summary of the incidence of clinical signs, body weights, food consumption, pathology findings and no clinical chemistry or hematology.

D. Dermal Studies

A subchronic dermal toxicity study was conducted in which DEF (99%) was applied topically to the shaved backs of 5 New Zealand white rabbits/sex/dose at 0, 2, 11 or 29 mg/kg/day (actual) for 6 hrs/day, 5 days/wk for 3 weeks (Sheets *et al.*, 1991). An additional 5 rabbits/sex were added to the control and high-dose group for a recovery study. Animals in the recovery groups were held for another 2 weeks after the last exposure. Clinical signs (muscle fasciculations, dried, cracked or flaking skin, erythema, tremors, decreased motor activity, anal stain, red conjunctiva, clear lacrimation, clear nasal discharge, edema, urine stain, and increased reactivity) were seen in both sexes at either 11 or 29 mg/kg/day. Muscle fasciculations were observed as early as day 2 in 9 of 10 rabbits at 29 mg/kg/day. Red conjunctiva, anal stains and lacrimation were also seen in a few animals at 2 mg/kg/day. The investigators attributed the red conjunctiva, lacrimation and anal stains to the plastic collars the rabbits wore during exposure to prevent licking of the application site. To support this conclusion, they noted that these signs resolved within one day in all of the recovery groups after the collars were removed. Although the investigators considered the incidence of anal stains to be treatment-related; the toxicological significance of this sign at 2 and 11 mg/kg/day is uncertain because the incidence was similar to the control group.

A reduction in mean body weights (M: 15%; F: 13%) and food consumption (M: 30%; F: 29%) was seen in both sexes at 29 mg/kg/day by study termination. No compound-related effects were observed with the ophthalmological and gross pathological examinations. Clinical pathological findings included an increased number of segmented white blood cells, a decreased number of lymphocytes, and an increase in blood urea nitrogen (BUN) levels in animals at 29 mg/kg/day. The toxicological significance of the changes in hematological and clinical chemistry values is uncertain without accompanying histological changes. The mean plasma ChE activity was reduced at 2 mg/kg/day (M: 82%; F: 89% of controls), 11 mg/kg/day (M: 43%; F: 46% of controls), and 29 mg/kg/day (M: 27%; F: 26% of controls). A reduction in the mean erythrocyte ChE activity was also observed at 2 mg/kg/day (M: 89%; F: 80% of controls), 11 mg/kg/day (M&F: 30% of controls), and 29 mg/kg/day (M: 28%; F: 20% of controls). The mean brain ChE activity was significantly reduced at 11 mg/kg/day (M: 86%; F: 85% of controls) and 29 mg/kg/day (M: 68%; F: 62% of controls). Microscopic findings were limited to acanthosis and

hyperkeratosis, which were observed in the skin at the dosing site of both sexes at 11 and 29 mg/kg/day. The hyperkeratosis was of minimal severity at 2 mg/kg/day and apparently reversible based on the decreased incidence in the high-dose-recovery group. Therefore, it was not considered toxicologically significant. Based on the muscle fasciculations at 29 mg/kg/day on day 2, the acute NOAEL for this study was 11 mg/kg/day. The subchronic NOAEL was 2 mg/kg/day based on muscle fasciculations, brain ChE inhibition (85-86% of controls), and microscopic lesions in the skin at 11 mg/kg/day. DPR toxicologists found this study acceptable based on the FIFRA guidelines.

E. Conclusions

The clinical signs observed with subchronic exposure to DEF were also primarily cholinergic signs. Unlike the acute toxicity studies, ChE inhibition data were available for most of the subchronic studies. ChE inhibition was the only possible adverse effect reported in the oral subchronic studies for DEF. With subchronic inhalation exposure, cholinergic signs, inflammatory changes in the lung, fatty droplets in the adrenal cortex, impaired retinal function, and pale retinal fundus were observed at the same dose level that produced significant brain ChE inhibition (60-75% of control activity). In the 3-week dermal toxicity study, muscle fasciculations and microscopic lesions in the skin at the dosing site were observed at dose levels that produced slight brain ChE inhibition (85% of control activity).

The lowest subchronic NOAEL with inhalation exposure was 7 mg/m^3 (1.7 mg/kg/day) based on brain ChE inhibition (73% of control activity), mild cholinergic signs, and inflammation in the lungs. However, this study had major deficiencies including an inadequate exposure period (3 weeks). A slightly higher subchronic NOAEL of 12.2 mg/m^3 (2.9 mg/kg/day) was established in an acceptable 13-week inhalation study based on clinical signs, brain ChE inhibition (60% of control activity), impaired retinal function, pale retinal fundus, and fatty droplets in the adrenal cortex. An acute NOAEL of 12.2 mg/m^3 (2.9 mg/kg) was also established in this study based on reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing and increased startle response that were observed within the first 3 days of exposure.

VI. CHRONIC TOXICITY

A. Introduction

Four chronic toxicity studies were available, including a 90-week mouse study, two 2-year rat studies, and a 1-year dog study. All four studies administered DEF to the animals in the diet. All of the studies met FIFRA guidelines, except one of the rat studies.

B. Oral Studies

In a 90-week study, 50 CD-1 mice/sex/group were fed DEF (98.6% purity) in the diet at 0, 10, 50 or 250 ppm (M: 0, 1.5, 8.4 or 48.1 mg/kg/day; F: 0, 2.0, 11.3 or 63.1 mg/kg/day) (Hayes, 1989). The survival rate was significantly reduced in both sexes at 250 ppm (M: 50%; F: 38%). The early deaths occurred primarily in the last five months, although there was a significant increase in deaths during months 12 to 18. Enlarged abdomens were seen in both sexes at 250 ppm during weeks 14 to 26. Paleness, loose stools and perineal staining were common in the 250 ppm animals in the second year and coincided with the period of increased mortality. The loose stools and perineal staining were not attributed to cholinesterase inhibition due to the late onset of these effects. Body weights increased for both sexes at 250 ppm after week 13. At necropsy, a significant increase in fluid-filled or dilated intestines and cecum was observed macroscopically in the 250 ppm animals. The males at 50 and 250 ppm had a significant increase in the incidence of an enlarged spleen. A significant increase in the absolute weights of the liver, spleen (males only) and heart (males only) was found in the 250 ppm animals. At study termination, the mean plasma ChE activity was significantly reduced at 10 ppm (M: 33%; F: 35% of controls), 50 ppm (M: 9%; F: 7% of controls), and 250 ppm (M: 6%; F: 3% of controls). The mean erythrocyte ChE activity was also reduced at 10 ppm (M&F: 82% of controls), 50 ppm (M: 58%; F: 63% of controls), and 250 ppm (M: 45%; F: 50% of controls). There was a statistically significant reduction in the mean brain ChE activity at 10 ppm (M: 91% of controls), 50 ppm (M: 87% of controls), and 250 ppm (M: 62%; F: 73% of controls). Both sexes at 250 ppm had evidence of anemia based on significant reductions in their mean erythrocyte count (M: 29%; F: 13%), hemoglobin (M: 18%; F: 13%) and hematocrit (M: 20%; F: 11%) values and increases in their mean corpuscular volume (M: 16%) and mean corpuscular hemoglobin (M: 20%) values at study termination. Females at 50 ppm also had significant reductions in their mean erythrocyte count (10%), hemoglobin (8%), and hematocrit (8%) values at study termination.

A significant increase in numerous non-neoplastic lesions in the intestines, liver, adrenal gland, and spleen were observed histologically in animals at 250 ppm (Tables 5 and 6). The

incidence of mucosal hyperplasia in the small intestine, dilated/edematous cecum or small intestine, necrosis/ulceration of the rectum, and adrenal degeneration/pigmentation were significant at 250 ppm with dose-related trends. The incidences of vacuolar degeneration in the small intestine and extramedullary hematopoiesis in the spleen (males only) were significant at 50 and 250 ppm with dose-related trends. The incidence of focal atypia (group of abnormal appearing cells) in the small intestine exhibited a dose-related trend, although the increase was not significant when compared with the concurrent controls. The study pathologist considered the focal atypia pre-neoplastic, although he made no comment about the mucosal hyperplasia which could also be considered pre-neoplastic. The study pathologist attributed the vacuolar degeneration to the inability of the epithelial cells to "absorb or secrete products", resulting in fluid accumulation. The extramedullary hematopoiesis in the spleen may be related to the anemia and enlarged spleen, but these findings were not usually present in the same animal at the same time. The increase in adrenal degeneration/pigmentation was considered by the study pathologist to be an enhancement of a common age-related lesion that may be due to stress. The incidence of liver hypertrophy exhibited a dose-related trend in both sexes, but was only significant in females at 250 ppm by pairwise statistical comparison to the concurrent controls. There was no correlation of the liver hypertrophy observed histologically with the increased liver weights.

The NOAEL was 10 ppm (M: 1.5 mg/kg/day; F: 2.0 mg/kg/day) based on vacuolar degeneration of the small intestine, spleen hematopoiesis, hematological changes, and brain ChE inhibition (87% of controls) at 50 ppm. Although statistically significant brain ChE inhibition was observed in males at 10 ppm, DPR toxicologists did not consider the reduction toxicologically significant for several reasons. First, the reduction at 10 ppm was only to 91% of control activity and no clinical signs were observed. Second, the reduction at 50 ppm was not much greater (87% of controls) and there were still no cholinergic signs. Finally, despite a reduction in the mean brain ChE activity of 62% and 73% of controls in males and females, respectively, at 250 ppm only mild cholinergic signs (loose stools and perineal staining) were observed. These findings indicate that the dose-response curve is not very steep and, therefore, the slight inhibition at 10 ppm is not of significant toxicological concern in establishing a NOAEL. DPR toxicologists found this study acceptable based on the FIFRA guidelines.

Table 5. Incidence of Non-neoplastic Microscopic Lesions in Male Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Vacuolar degeneration	0/50^{+++} (0%)	1/50 (2%)	8/50** (16%)	28/50*** (56%)
Mucosal hyperplasia	0/50^{+++} (0%)	0/50 (0%)	1/50 (2%)	22/50*** (44%)
Focal atypia	0/50^{+++} (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Dilated/distended	0/50^{+++} (0%)	0/50 (0%)	2/50 (4%)	7/50** (14%)
Cecum				
Dilated/edematous	4/50^{++} (8%)	8/50 (16%)	6/50 (12%)	13/50* (26%)
Rectum				
Necrosis/ulceration	0/45^{+++} (0%)	1/49 (2%)	1/47 (2%)	10/46*** (22%)
Liver				
Hypertrophy	1/50^{++} (2%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adrenal				
Degeneration/pigment.	17/50^{+++} (34%)	15/50 (30%)	21/50 (42%)	39/50*** (78%)
Spleen				
Hematopoiesis	6/50^{+++} (12%)	6/50 (12%)	14/50* (28%)	19/50** (38%)

a The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.

$^{++}$, $^{+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.01$, and 0.001, respectively (Peto *et al.*, 1980).

*, **, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

Table 6. Incidence of Non-neoplastic Microscopic Lesions in Female Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Vacuolar degeneration	0/50^{+++} (0%)	0/50 (0%)	11/50*** (22%)	28/50*** (56%)
Mucosal hyperplasia	1/50^{+++} (2%)	0/50 (0%)	0/50 (0%)	19/50*** (38%)
Focal atypia	0/50^{+} (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Dilated/distended	2/50^{+++} (4%)	0/50 (0%)	1/50 (2%)	18/50*** (36%)
Cecum				
Dilated/edematous	7/50^{+++} (14%)	3/50 (6%)	4/50 (8%)	20/50** (40%)
Rectum				
Necrosis/ulceration	2/50^{+++} (4%)	0/46 (0%)	1/50 (2%)	14/49** (29%)
Liver				
Hypertrophy	0/50^{+++} (0%)	2/50 (4%)	0/50 (0%)	6/50* (12%)
Adrenal				
Degeneration/pigment.	18/50^{+++} (36%)	26/50 (52%)	22/50 (44%)	38/49*** (78%)
Spleen				
Hematopoiesis	16/50 (32%)	14/50 (28%)	18/50 (36%)	20/50 (40%)

a The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.

$^{+}$, $^{+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*, **, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

Groups of 24 Sprague-Dawley rats/sex/group were fed DEF (97.7%) in the diet at 0, 5, 25, 100 or 250 ppm (0, 0.25, 1.25, 5.0 or 12.5 mg/kg/day)[6] for 2 years (Root *et al.*, 1967). There was no effect on survival even at the highest dose level. The mean body weight gain in males at 100 ppm was reduced (~12% relative to controls) by the end of the study. The females fed DEF at 250 ppm also had significantly reduced (~20% relative to controls) mean weight gain. The mean plasma ChE activity was significantly reduced at 25 ppm (M: 69%; F: 65% of controls), 100 ppm (M: 31%; F: 26% of controls), and 250 ppm (M: 22%; F: 18% of controls). A reduction in mean erythrocyte ChE activity was seen at 25 ppm (M: 53%; F: 42% of controls), 100 ppm (M: 27%; F: 18% of controls), and 250 ppm (M: 15%; F: 12% of controls). The mean brain ChE activity was significantly reduced at 100 ppm (F: 69% of controls) and 250 ppm (M: 57%; F: 32% of controls). The NOAEL was 25 ppm (1.25 mg/kg/day) based on the liver cytoplasmic vacuolation, reduced weight gain and brain ChE inhibition. This study had major deficiencies including incomplete histopathological examination, no hematology or clinical chemistry data, no analysis of dosing material, no individual data, and intercurrent disease.

A combined chronic toxicity/oncogenicity/neurotoxicity study was conducted in which Fischer 344 rats were fed DEF (98.5%) in the diet at 0, 4, 40 or 320 ppm (M: 0, 0.2, 1.8 or 16.8 mg/kg/day; F: 0, 0.2, 2.3 or 21.1 mg/kg/day) for 2 years (Christenson, 1992). Fifty rats/sex/dose were assigned to the oncogenicity study, 20 rats/sex were assigned to the control and 320 ppm groups as interim sacrifice animals for the chronic toxicity study, and 20 rats/sex/dose were assigned to the neurotoxicity study. The incidences of a number of clinical signs were higher in the 320 ppm rats, including pale eye, ocular opacity, rough coat, rash, raised zone of the skin, urine stain, clear discharge (origin not reported), soft feces, and diarrhea. The mean body weight gains were reduced in both sexes at 320 ppm (~15%) at study termination. Body temperature reductions occurred more frequently in the 40 and 320 ppm rats, although not in a consistent or dose-related manner.

Significant decreases in several hematological values (erythrocyte counts, hemoglobin, hematocrits, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration) were found in blood drawn from the 40 and 320 ppm rats at 6 and 12 months, but by 18 and 24 months some of these values had returned to normal levels. In fact, the erythrocyte count, hemoglobin and hematocrit values had actually increased in the 320 ppm rats compared to controls, possibly from some compensatory mechanism(s). These hematological changes were considered an adverse effect based on the evidence in hens that DEF is hydrolyzed in the gut to nBM which can cause methemoglobinemia and eventual cell lysis (Abou-Donia, 1979: Abou-

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[6] Estimated assuming that for a rat 1 ppm in the diet is equivalent to 0.05 mg/kg/day (FDA, 1959)

Donia *et al.*, 1979a&b). The NOAEL for the hematological changes was 4 ppm (M & F: 0.2 mg/kg/day). Several clinical chemistry values, including a decrease in plasma glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total protein, albumin, and globulin and an increase in BUN, triglycerides, and creatine kinase (CK), were also significantly different in the 40 and 320 ppm groups compared to controls at 6 months. A few of these values had also returned to control levels at both dose levels by study termination, including AST, ALT, CK, and triglycerides. Other values had only returned to control levels in the 40 ppm group (total protein, albumin, globulin, and BUN). The toxicological significance of these changes in clinical chemistry values is uncertain, especially in absence of any histological changes in the liver, kidney or heart. There was a reduction in the mean plasma ChE activity at 4 ppm (M: 84%; F: 94% of controls), 40 ppm (M: 44%; F: 40% of controls), and 320 ppm (M: 20%; F: 17% of controls) at study termination. The mean erythrocyte ChE activity was reduced at 40 ppm (M: 73%; F: 72% of controls) and 320 ppm (M: 52%; F: 53% of controls) while the mean brain ChE activity was only reduced in the 320 ppm rats (M: 40%; F: 32% of controls). The NOAEL for brain ChE inhibition was 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day).

Ophthalmologic examination revealed an increased incidence of cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis and/or uveitis in both sexes at 320 ppm at study termination (Tables 7 and 8). These effects were not seen in the 1-year interim sacrifice animals. An increased incidence of bilateral unrecordable (flat) ERG responses was seen in 2-year old rats of both sexes at 320 ppm. Microscopic examination of the eye also revealed bilateral retinal atrophy (1- and 2-year) and optical nerve atrophy (2-year) in both sexes at 320 ppm. Because the cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis/uveitis, and optical nerve atrophy were not seen in the one-year rats, the study pathologist concluded that these effects were secondary to the retinal atrophy. The NOAEL for ocular lesions was 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day).

No dose-related increases were found in the microscopic lesions of the brain, spinal cord and sciatic nerve of rats assigned to the neurotoxicity study. Some studies suggest that rodents (especially Fischer 344 rats) are less sensitive to OPIDN (Abou-Donia, 1981; Somkuti *et al.*, 1988; De Bleeker *et al.*, 1992). The susceptibility of rodents to OPIDN appears to be variable based on studies by other investigators (Padilla and Veronesi, 1988; Veronesi *et al.*, 1991; Moretto *et al.*, 1992; Inui *et al.*, 1993). Differences in age, regeneration of peripheral nerves, aging and resynthesis of NTE, and metabolism have been suggested as possible explanations for the variable response among rodents (Moretto *et al.*, 1992; Veronesi *et al.*, 1991). Since chemicals that produce OPIDN can affect both sensory and motor nerves (Abou-Donia, 1981), it

Table 7. Incidence of Ophthalmologic and Microscopic Lesions in Male Rats Fed DEF for 2 Years

	Dose Level (ppm)			
	0	4	40	320
Ophthalmology Examination				
Posterior, subcapsular or complete cataract	5/36^{+++} (14%)	4/30 (13%)	5/36 (14%)	27/32*** (84%)
Lens Opacity	6/36 (17%)	4/30 (13%)	3/36 (8%)	8/32 (25%)
Diffuse or focal corneal opacity	21/36^{+++} (58%)	20/30 (67%)	26/36 (72%)	31/32*** (97%)
Corneal neovascularization	2/36^{+++} (5%)	6/30 (20%)	1/36 (3%)	15/32*** (47%)
Iritis and/or uveitis	3/36^{+++} (8%)	5/30 (17%)	7/36 (19%)	31/32*** (97%)
Electroretinographic Examination				
Bilateral unrecordable responses	0/15^{+++} (0%)	2/9 (22%)	0/15 (0%)	11/13*** (85%)
Microscopic Examination				
Bilateral retinal atrophy	1/50^{+++} (2%)	0/50 (0%)	0/50 (0%)	50/50*** (100%)
Optic nerve atrophy	10/50^{+++} (20%)	6/50 (12%)	6/50 (12%)	32/50*** (64%)
Small intestine				
Vacuolar degeneration	0/50^{+++} (0%)	1/50 (2%)	24/50*** (48%)	37/50*** (74%)
Hyperplasia	0/50^{+++} (0%)	3/50 (6%)	23/50*** (46%)	34/50*** (68%)
Adrenal vacuolar degeneration	6/50^{+++} (12%)	6/49 (12%)	9/50 (18%)	35/49*** (71%)

$^{+,+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

Table 8. Incidence of Ophthalmologic and Microscopic Lesions in Female Rats Fed DEF for 2 Years

	Dose Level (ppm)			
	0	4	40	320
Ophthalmology Examination				
Posterior, subcapsular or complete cataract	4/41^{+++} (10%)	6/38 (16%)	6/34 (18%)	15/32*** (47%)
Lens Opacity	9/41^{+++} (22%)	8/38 (21%)	5/34 (15%)	20/32*** (62%)
Diffuse or focal corneal opacity	20/41^{+++} (49%)	27/38* (71%)	20/34 (59%)	31/32*** (97%)
Corneal neovascularization	11/41^{+++} (27%)	7/38 (18%)	4/34 (12%)	19/32** (59%)
Iritis and/or uveitis	3/41^{+++} (7%)	5/38 (13%)	5/34 (15%)	29/32*** (91%)
Electroretinographic Examination				
Bilateral unrecordable responses	1/16^{+++} (6%)	2/16 (12%)	0/13 (0%)	7/8*** (88%)
Microscopic Examination				
Bilateral retinal atrophy	0/50^{+++} (0%)	2/50 (4%)	0/50 (0%)	40/50*** (80%)
Optic nerve atrophy	10/50^{+++} (20%)	6/50 (12%)	6/50 (12%)	32/50*** (64%)
Small intestine				
Vacuolar degeneration	0/50^{+++} (0%)	0/50 (0%)	19/50*** (38%)	35/50*** (70%)
Hyperplasia	1/50^{+++} (2%)	0/50 (0%)	11/50** (22%)	30/50*** (60%)
Adrenal vacuolar degeneration	10/50^{+++} (20%)	6/50 (12%)	16/50 (32%)	41/50*** (82%)

$^{+,+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*,**,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

is possible that the degeneration of the retina and optic nerve observed in this study is another, perhaps more sensitive, sign of OPIDN in rats.

There was an increase in other non-ocular lesions, including vacuolar degeneration of the adrenal glands and small intestine and hyperplasia of the small intestine. The incidence of the vacuolar degeneration and hyperplasia of the small intestine was increased in both sexes at 40 and 320 ppm at the terminal sacrifice. The vacuolar degeneration of the small intestine was also seen in both sexes at the 1-year sacrifice. The incidence in 1-year-old males was 0/20, 0/10, 7/10, and 18/20 at 0, 4, 40, and 320 ppm, respectively. The incidence in 1-year-old females was 0/20, 0/10, 8/10, and 16/20 at 0, 4, 40, and 320 ppm, respectively. The lesions in the small intestine correlated with the gross findings of thickened and white discoloration. The vacuolar degeneration of the adrenal glands was increased in 2-year old rats of both sexes at 320 ppm. The adrenal lesions correlated with the gross finding of enlargement and increased adrenal weights. The NOAELs for the lesions in the small intestine and adrenal glands were 4 ppm (M & F: 0.2 mg/kg/day) and 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day), respectively. A decrease in the incidence of chronic nephropathy was seen in the 2-year-old rats. The incidence among males was 50/50, 50/50, 46/50, and 34/50 at 0, 4, 40, and 320 ppm. The incidence among females was 39/50, 45/50, 30/50, and 25/50 at 0, 4, 40, and 320 ppm, respectively. The overall NOAEL for this study was 4 ppm (M & F: 0.2 mg/kg/day) based on the mucosal hyperplasia and vacuolar degeneration of the small intestine, and hematological changes. This study was acceptable to DPR toxicologists based on FIFRA guidelines.

In a chronic dog study, 4 beagle dogs/sex/group were administered DEF (98.5%) in the feed at 0, 4, 16 or 64 ppm (M: 0, 0.1, 0.4 or 1.7 mg/kg/day; F: 0, 0,1, 0.4 or 2.0 mg/kg/day) for 1 year (Christenson, 1991). There were no treatment-related differences in body weights, food consumption, clinical signs, clinical chemistry, brain cholinesterase, urinalysis, palpable masses, gross pathologic, histopathologic and ophthalmologic lesions. At study termination, the mean plasma ChE activity was significantly depressed at 16 ppm (M: 67% of controls) and 64 ppm (M: 38%; F: 52% of controls). The mean erythrocyte ChE activity was also reduced (M: 87%; F: 84% of controls) at 64 ppm. Slight reductions in the mean erythrocyte count (9-14%), hemoglobin value (6-13%), and hematocrit (8-12%) were observed in females at 64 ppm on days 91, 182, 273, and 364. Although the reductions in the means were greatest on day 364, the differences were only statistically significant on day 273. The NOAEL was 16 ppm (0.4 mg/kg/day) based on the hematological changes in females. This study was acceptable to DPR toxicologists based on the FIFRA guidelines.

C. Conclusions

Mice, rats, and dogs exposed to DEF orally for one year or longer all had evidence of marked anemia based on reduced hematological values. Significant brain ChE inhibition was observed in all three species. Gastrointestinal effects were seen in both mice and rats including vacuolar degeneration and hyperplasia of the small intestine. Degeneration or pigmentation of the adrenal glands were also seen in both mice and rats. Liver effects were also noted in both species (hypertrophy in mice and cytoplasmic vacuolation in rats). Other treatment-related histopathological changes were only seen in one species. Extramedullary hematopoiesis was observed in the spleen of mice. Ocular lesions were also seen in rats, including cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis/uveitis, bilateral unrecordable ERG responses, bilateral retinal atrophy, and optical nerve atrophy. The lowest NOAEL was 4 ppm (0.2 mg/kg/day) based on mucosal hyperplasia and vacuolar degeneration of the small intestine and anemia in rats.

VII. ONCOGENICITY

A. Introduction

In two of the studies described under the chronic toxicity section, animals were also examined for oncogenic effects. One of these studies was conducted using mice and the other using rats. Both studies met FIFRA guidelines for oncogenicity studies.

B. Oral Studies

In a 90-week study, 50 CD-1 mice/sex/group were fed DEF (98.6% purity) in the diet at 0, 10, 50 or 250 ppm (M: 0, 1.5, 8.4 or 48.1 mg/kg/day; F: 0, 2.0, 11.3 or 63.1 mg/kg/day) (Hayes, 1989). A significant increase in several neoplastic lesions was reported in mice fed DEF in the diet at the high dose (Tables 9 and 10) (Hayes, 1989). There was a significant increase in liver hemangiosarcomas in males at 250 ppm that exhibited a dose-related trend. Among the animals with liver hemangiosarcomas most also had hemorrhage and/or necrosis in the liver (M - 0/1, 1/1, 4/4, 6/7; F - 2/2, ½, 2/2, 1/1). The incidence of liver hemangiosarcomas in the males at 250 ppm was outside the historical control range for males reported by this laboratory (0-6%). However, the historical control data consisted of only three studies with 50 mice/sex/study.

There was an increase in adenocarcinomas of the small intestine in both sexes which were significant by trend analysis primarily due to the response at the high-dose. The increase in these tumors was highly significant by pairwise comparison with concurrent controls in males ($p < 0.001$), but not in females at 250 ppm ($p = 0.054$). Some of these tumors were associated with inflammatory responses. The reported historical control range for this laboratory was 0% for both sexes. In addition, numerous non-neoplastic lesions were seen in the small intestine of both sexes in this study which were previously described under chronic toxicity (Tables 5 and 6). Mice with adenocarcinomas often had focal atypia, mucosal hyperplasia, and/or vacuolar degeneration of the small intestines, too. The investigators suggested that these lesions in the small intestine are interrelated based on their multiplicity and dose relationship.

The incidence of alveolar/bronchiolar adenomas was also significantly higher in females at 250 ppm and were significant by trend analysis essentially due to the response at the high dose. The incidence at the high dose was outside the laboratory's historical control range for these tumors in females (0-14%). There was also a significant positive trend in other potentially pre-neoplastic lesions in the lungs of females including epithelialization (0/50, 1/50, 4/50, 5/50)

Table 9. Incidence of Neoplastic Microscopic Lesions in Male Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Adenocarcinoma[b]	0/47^{+++} (0%)	0/48 (0%)	0/47 (0%)	9/46*** (20%)
Liver				
Hemangiosarcoma[c]	1/47^{++} (2%)	1/48 (2%)	4/47 (9%)	7/46* (15%)
Lungs				
Alveolar/bronchiolar adenoma[d]	11/47 (23%)	9/48 (19%)	5/47 (11%)	9/46 (20%)
Alveolar/bronchiolar carcinoma[e]	3/47 (6%)	5/48 (10%)	4/47 (9%)	3/46 (7%)
Alveolar/bronchiolar tumors - combined	11/47 (23%)	13/48 (27%)	9/47 (19%)	11/46 (24%)

a The denominator is the number of animals at risk (excluding those that died before the first tumor was observed or 52 weeks, whichever came first); the number in parentheses represents the incidence in percentage.
b First small intestine adenocarcinoma observed on week 75 at 250 ppm.
c First liver hemangiosarcoma observed on week 59 at 50 ppm.
d First alveolar/bronchiolar adenoma observed on week 57 at 10 ppm.
e First alveolar/bronchiolar carcinoma observed on week 57 10 ppm.
$^{++}$, $^{+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.01$ and 0.001, respectively.
*,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

and focal hyperplasia (3/50, 4/50, 3/50, 8/50). The increase in epithelialization was significant at 250 ppm. The multiplicity of the lung lesions (focal hyperplasia and alveolar/bronchiolar adenomas and carcinomas) was elevated in the females at 250 ppm (0/50, 0/50, 1/50, 9/50).

Small intestine adenocarcinomas and liver hemangiosarcomas were present in several males at 250 ppm that died during the study (M - 1/16, 0/14, 3/21, 12/30; F - 1/19, 1/17, 2/22, 2/31) and may account for some of the early deaths. There was no association with the early deaths and the tumor incidence in females. The liver hemangiosarcomas, small intestine

Table 10. Incidence of Neoplastic Microscopic Lesions in Female Mice Fed DEF for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Adenocarcinoma[b]	0/49[++] (0%)	1/45 (2%)	0/44 (0%)	4/47[c] (9%)
Liver				
Hemangiosarcoma[d]	2/49 (4%)	2/47 (4%)	2/47 (4%)	1/48 (2%)
Lungs				
Alveolar/bronchiolar adenoma[e]	5/49[+++] (10%)	5/45 (11%)	2/44 (5%)	15/47** (32%)
Alveolar/bronchiolar carcinoma[f]	1/49 (2%)	2/45 (4%)	0/44 (0%)	2/47 (4%)
Alveolar/bronchiolar tumors - combined	6/49[+++] (12%)	7/45 (16%)	2/44 (5%)	16/47** (34%)

a The denominator is the number of animals at risk (excluding those that died before the first tumor was observed or 52 weeks, whichever came first); the number in parentheses represents the incidence in percentage.
b First small intestine adenocarcinoma observed on week 69 at 250 ppm.
c Not significantly different ($p = 0.054$) from the control group based on Fisher's exact test.
d First liver hemangiosarcoma observed on week 46 at 50 ppm.
d First alveolar/bronchiolar adenoma observed on week 74 at 250 ppm.
e First alveolar/bronchiolar carcinoma observed on week 75 at 250 ppm.
++, +++ Significant trend based on a dose-weighted chi-square test at $p < 0.01$ and 0.001, respectively.
** Significantly different from the control group based on the Fisher's exact test at $p < 0.01$.

adenocarcinomas and alveolar/bronchiolar adenomas were first seen in females on week 46 (50 ppm), 69 (250 ppm), and 74 (250 ppm), respectively.

There was no dose-related increase in the incidence of benign or malignant tumors in the combined chronic toxicity/oncogenicity/neurotoxicity study in Fischer 344 rats fed DEF in the diet at 0, 4, 40 or 320 ppm for 2 years (Christenson, 1992).

C. Conclusions

There was no evidence of oncogenicity in the rat study; however, in mice there was a significant increase in adenocarcinomas of the small intestine in both sexes, liver hemangiosarcomas in males, and alveolar/bronchiolar adenomas in females. Not only was there an increase in tumors at more than one site, but there was a significant increase in one tumor type (adenocarcinomas of the small intestine) in both sexes by trend analysis.

VIII. GENOTOXICITY

A. Introduction

Five genotoxicity tests were available for DEF including an Ames assay, an *in vitro* chromosomal aberrations assay with Chinese hamster ovary cells, two *in vitro* sister chromatid exchange assay with Chinese hamster V79 cells, and an unscheduled DNA synthesis assay with rat primary hepatocytes. Three of these tests met FIFRA guidelines (which refer to the Toxic Substances Control Act (TSCA) guidelines for genotoxicity studies).

B. Gene Mutation

DEF (98.5%) did not produce an increase in the mutation frequency in a mutagenicity assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations ranging from 667 to 10,000 µg/plate with and without metabolic activation (Curren and Gentry, 1989). The assay was acceptable to DPR toxicologists based on the FIFRA guidelines.

C. Chromosomal Aberrations

No increase in chromosomal aberrations was seen in Chinese hamster ovary cells exposed to DEF (98.5%) at concentrations of 0.007 to 0.1 µl/ml with metabolic activation and at 0.004 to 0.05 µl/ml without activation (Putman and Morris, 1989). This study was acceptable to DPR toxicologists based on FIFRA guidelines.

D. Other Genotoxic Effects

Chen *et al.* (1982a&b) found no increase in sister chromatid exchanges in Chinese hamster V79 cells exposed to DEF (95.7%) at concentrations from 2.5 to 20 µg/ml with and without metabolic activation. Nicholas and Van Den Berghe (1982) also reported no increase in sister chromatid exchanges in Chinese hamster V79 cells exposed to DEF at concentrations up to 60 µM (18.9 µg/ml) without metabolic activation.

In an unscheduled DNA synthesis assay, no increase in the average grains per nucleus were observed in rat primary hepatocytes exposed to DEF (98.5%) at concentrations between 0.0001 and 0.03 µl/ml (Curren, 1989). DPR toxicologists found this study acceptable based on FIFRA guidelines.

E. Conclusions

There was no evidence of genotoxicity in any of the five available studies.

IX. REPRODUCTIVE TOXICITY

A. Introduction

Only one reproductive toxicity study was available for DEF. The test compound was administered to rats by the oral route in this study. The study met FIFRA guidelines.

B. Oral Studies

In a two-generation rat reproduction study, 30 Sprague Dawley rats/sex/group/generation were fed DEF (98.5%) in the diet at 0, 4, 32 or 260 ppm (M: 0, 0.3, 2.2 or 19.1; F: 0, 0.4, 3.0 or 24.7 mg/kg/day) for 10 weeks/generation prior to mating (Eigenberg, 1991a). Body weight gains were significantly lower during all phases of the study in F_0 dams and during lactation in F_1 dams at 260 ppm. The mean body weights were significantly reduced for the F_{1a} pups at 260 ppm from birth (11%) through lactation (21-29%). The investigators found in a subsequent cross-fostering study that the low birth weights were not due to a compound-related effect on development, but rather a weight loss that occurred between birth and the time the birth weights were taken which was up to 24 hours later (Eigenberg, 1991b). There was no difference in the mean body weights for the F_{2a} pups at birth, but by day 4 the mean body weight at 260 ppm was reduced by 9%. The mean pup weights were also significantly reduced at 260 ppm on days 7, 14, and 21 (14-22%). Maternal food consumption was reduced in both generations at 260 ppm. Tremors were observed in one F_0 dam at 260 ppm and abnormal head tilt was seen in three F_1 dams at 260 ppm. Clinical signs observed in F_{1a} and F_{2a} pups at 260 ppm included cannibalization, bite marks, bruised body, diffuse purple discoloration on head, shoulders and abdomen, dehydration, unkempt appearance and moribundity. The investigators attributed the increased cannibalization, bite marks and bruised bodies to some unknown effect of DEF on the dams based on a supplemental cross-fostering study (Eigenberg, 1991b).

Several reproductive parameters were affected at 260 ppm (Table 11). There was a noticeable reduction in the fertility index in the F_1 generation (76% vs. 97% for controls), although it was not statistically significant. This effect was considered toxicologically significant based on a supplemental study in which a similar reduction (83% vs. 90% for controls) was observed in the F_1 generation at 260 ppm (Eigenberg, 1991c). There was a significant increase in gestation length in the F_{2a} litters at 260 ppm which was reproduced in a supplemental cross fostering study (Eigenberg, 1991b). There were also significant reductions in the birth index, live birth index, and viability index in both generations at 260 ppm. The

Table 11. The Reproductive Effects of DEF in a Two-Generation Rat Study

Reproductive Effect	Generation	Dose Level (ppm) 0	4	32	260
Fertility Index	F_0	90	97	90	90
	F_1	97	93	90	76
Mean Gestation Length (days)	F_{1a}	21.8	22.0	21.9	22.2
	F_{2a}	21.9	22.0	22.0	22.4*
Birth Index	F_{1a}	91	89	90	77*
	F_{2a}	92	91	92	87*
Live Birth Index	F_{1a}	100	97	100	80*
	F_{2a}	99	95	100	87*
Viability Index (day 4)	F_{1a}	96	96	100	90*
	F_{2a}	97	100	97	81*
(day 21)	F_{1a}	100	99	99	83*
	F_{2a}	100	99	100	90*
Mean Pup Weight (g) (day 4)	F_{1a}	7.1	7.1	7.2	6.3*
	F_{2a}	6.8	7.2*	7.0	6.7
(day 21)	F_{1a}	49.5	50.2	50.1	35.2*
	F_{2a}	49.1	49.5	50.2	38.3*

* Significantly different from the control group by the Kruskal-Wallis and Mann-Whitney U test ($p < 0.05$).

reductions in the birth index and the live birth index were probably related to maternal toxicity. Based on the cross-fostering study, the reductions in the neonatal pup weights and the viability index were also probably due to some unknown effect of DEF on the dams (Eigenberg, 1991b).

Cholinesterase activity was measured in adults at week 8 of premating for each generation and in both adults and pups at the terminal sacrifice. At week 8 of premating, there were significant reductions in the mean plasma ChE activity at 32 ppm (F_0M: 67%; F_0F: 32%; F_1F: 41% of controls) and 260 ppm (F_0M: 30%; F_1M: 43%; F_0F: 9%; F_1F: 7% of controls). At the terminal sacrifice, a significant reduction in the mean plasma ChE activity was observed at 4 ppm (F_0F: 75% of controls), 32 ppm (F_0M: 82%; F_0F&F_1F: 28% of controls) and 260 ppm (F_0M: 22%; F_1M: 32%; F_0F: 10%; F_1F: 7% of controls). At week 8, there were significant reductions in the mean

erythrocyte ChE activity at 4 ppm (F_1M: 91% of controls), 32 ppm (F_0M: 65%; F_1M: 74%; F_0F: 63%; F_1F: 72% of controls), and 260 ppm (F_0M: 50%; F_1M: 57%; F_0F: 51%; F_1F: 55% of controls). At the terminal sacrifice, the mean erythrocyte ChE activity was also significantly reduced at 4 ppm (F_0F: 88%; F_1F: 93% of controls), 32 ppm (F_0M: 69%; F_1M: 72%; F_0F: 54%; F_1F: 51% of controls) and 260 ppm (F_0M: 47%; F_1M: 61%; F_0F: 48%; F_1F: 47%). The mean brain ChE activity was reduced only at 32 ppm (F_0F&F_1F: 71% of controls) and 260 ppm (F_0M: 63%; F_1M: 67%; F_0F&F_1F: 19% of controls). There were gender-related differences in ChE activity which were most pronounced at the terminal sacrifice. One explanation for these differences was the higher compound consumption in females during lactation. During lactation, the average compound consumption for females in both generations was approximately twice as high as their consumption during premating and gestation (0.7, 5.5, and 39.2 mg/kg/day at 4, 32, and 260 ppm, respectively). Significant reductions in the mean plasma ChE activity were observed in 21-day-old pups at 260 ppm (F_{1a}M: 64%; F_{2a}M: 49%; F_{1a}F: 62%; F_{2a}F: 36% of controls). The mean erythrocyte ChE activity was also significantly reduced (F_{2a}M: 75%; F_{1a}F: 77%; F_{2a}F: 62% of controls). There was also a significant reduction in the mean brain ChE activity in 21-day-old F_{2a} pups at 260 ppm (M&F: 85% of controls).

There were no apparent compound-related increases in gross pathological findings in the adults. Sporadic gross ocular lesions (discoloration, opacity, reduced size, abnormal texture and enlargement) were observed in all groups of F_0 and F_1 adults which were attributed to the orbital bleeding technique by the investigator. Possible compound-related retinal degeneration was observed microscopically in two females (one at 4 ppm and the other at 260 ppm) with gross ocular lesions (corneal opacity and enlargement, respectively). The eyes were examined in only a few rats with gross ocular lesions, probably because the effect of DEF on the retina was not known when this study was conducted. Consequently, a dose-response was not apparent. In the pups, possible compound related effects observed at 260 ppm included cannibalism, discolored livers, uninflated lungs (stillbirths) and empty stomachs (non-suckling).

The reproductive NOAEL was 32 ppm (F: 3.0 mg/kg/day) based on the reduction in the fertility, birth, live birth and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, and gross pathological lesions in pups. The parental NOAEL was 4 ppm (0.4 mg/kg/day) based on the reduced brain ChE activity (71% of control activity) in F_0 and F_1 females at 32 ppm. This study was considered acceptable by DPR toxicologists based on FIFRA guidelines.

C. Conclusions

Several reproductive effects were seen in the one rat reproductive study. The reproductive effects included reductions in the fertility, birth, and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, and gross pathological lesions in pups. The reproductive NOAEL was 32 ppm (F: 3.0 mg/kg/day). Other non-reproductive effects that were observed included brain ChE inhibition and reduced body weight gains. The parental NOAEL was 4 ppm (0.4 mg/kg/day) based on the brain ChE inhibition. Based on the cross-fostering study, the reproductive effects in pups, such as reduced birth and viability indices, reduced neonatal weights, clinical signs and gross pathological lesions, are probably related to maternal toxicity rather than a direct effect of DEF on the pups.

X. DEVELOPMENTAL TOXICITY

A. Introduction

Two teratology studies were conducted with DEF, one in rats and the other in rabbits. Both studies administered DEF by oral gavage. These studies met FIFRA guidelines. In addition, an inhalation teratology study was conducted in which mice and rats were exposed to vapors of nBM. This study did not meet FIFRA guidelines.

B. Oral Studies

In a teratology study, DEF (98%) was administered to 33 mated female Sprague-Dawley rats/group by oral gavage in 0.5% carboxymethylcellulose at 0, 1, 7 or 28 mg/kg/day on gestation days 6 to 15 (Kowalski *et al.*, 1 986). Excessive salivation was observed in two dams at 28 mg/kg/day on treatment days 3 and 6 (gestation days 9 and 12). There was a significant reduction in the mean body weight gain of the 28 mg/kg/day group. The mean plasma and erythrocyte ChE activity in the dams was significantly reduced at 7 mg/kg/day (42 and 29% of controls, respectively) and 28 mg/kg/day (25 and 13% of controls, respectively) on day 16. Although the mean maternal brain ChE activity remained significantly reduced (54% of controls) on day 20 at 28 mg/kg/day, fetal brain ChE activity was unaffected. No treatment-related teratogenic or other developmental effects were seen. The maternal NOAEL was 7 mg/kg/day based on salivation, brain ChE inhibition (54% of controls), and reduced body weight gain. The developmental NOAEL was greater than or equal to 28 mg/kg/day, the highest dose tested. This was an acceptable study to DPR toxicologists based on FIFRA guidelines.

Groups of 17 mated female American Dutch rabbits were given DEF (98%) by oral gavage in carboxymethylcellulose at 0, 1, 3, or 9 mg/kg/day on days 7 to 19 of gestation (Clemens *et al.*, 1987). Although control animals gained 150 g on average from gestation days 7 to 21, animals at 9 mg/kg/day gained no weight on average during this time. The animals at 9 mg/kg/day also tended to consume less food during the treatment period, although the difference was not statistically significant. The mean plasma ChE activity was significantly reduced at 1 mg/kg/day (60% of controls), 3 mg/kg/day (46% of controls), and 9 mg/kg/day (33% of controls) on day 20. There was also a significant reduction in erythrocyte ChE activity at 1 mg/kg/day (30% of controls), 3 mg/kg/day (15% of controls), and 9 mg/kg/day (7% of controls) on day 20. The slight reduction in the mean brain ChE activity (95% of controls) at 9 mg/kg/day was not statistically significant. There was no treatment-related increase in embryotoxicity, fetal malformations or variations. The maternal NOAEL was 3 mg/kg/day based on no body weight

gain during exposure. The developmental NOAEL was greater than or equal to 9 mg/kg/day, the highest dose tested. DPR toxicologists found this study acceptable based on FIFRA guidelines.

C. N-Butyl Mercaptan

In an inhalation teratology study, 25 mice/dose were exposed to vapors of nBM (97.5%) at 0, 10, 68 or 152 ppm (actual; 0, 17, 113 or 252 mg/kg/day)[7] for 6 hr/day during gestation days 6-16 (Thomas *et al.*, 1987). Seventeen mice at 68 and 152 ppm died. One dam at 152 ppm had limb paralysis and spasmodic respiratory appearance. Emaciation, unkempt appearance, lethargy and red/brown perianal staining were seen in dams at 68 and 152 ppm. There was a reduction in the terminal maternal body weights (>10%) and an increase in postimplantation losses at 68 and 152 ppm. The total number of fetuses with malformations (which included cleft palate, open eye, exencephaly, hydrocephaly, vertebral anomalies and bent bones) was also significantly higher at 68 ppm, although there was no significant difference in the number of malformations/group on a litter basis. Four of the 5 fetuses with cleft palate at 68 ppm occurred in two litters in which there was evidence of both maternal and fetal toxicity based on maternal weight loss and lower fetal weights. The maternal NOAEL for nBM was 10 ppm (17 mg/kg/day) based on the mortality, reduced weight gain and clinical signs. The developmental NOAEL was also 10 ppm based on the increased postimplantation losses and malformations. This study was only available as a published report and, therefore, it is not known if it met FIFRA guidelines.

Thomas *et al.* (1987) also exposed 25 rats/dose to vapors of nBM (97.5%) at 0, 10, 68 or 152 ppm (actual; 0, 9, 60 or 135 mg/kg/day)[8] for 6 hr/day during gestation days 6-19 (Thomas *et al.*, 1987). There were no mortalities or significant treatment-related clinical signs. There was no evidence of developmental toxicity. The maternal and developmental NOAELs in rats were greater than 152 ppm (135 mg/kg/day), the highest dose tested.

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[7] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a mouse was assumed to be 0.45 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979). A 100% respiratory retention and absorption was assumed.

[8] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a rat was assumed to be 0.24 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979). A 100% respiratory retention and absorption was assumed.

D. Conclusions

No treatment-related increases in embryotoxicity, fetal malformations or variations were observed in rats and rabbits exposed to DEF by the oral route. Maternal effects included salivation, brain ChE inhibition, and reduced body weight gain. The maternal NOAELs were 7 and 3 mg/kg/day for rats and rabbits, respectively. An increased post-implantation loss was observed in mice exposed to vapors of nBM. The total number of malformations was also higher, although not on a litter basis. The developmental NOAEL for nBM was 10 ppm (17 mg/kg/day). The maternal effects included increased mortalities, reduced body weight gain, and clinical signs. The maternal NOAEL for nBM in mice was also 10 ppm. There were no treatment-related increases in developmental or maternal effects in rats exposed to vapors of nBM.

XI. NEUROTOXICITY

A. Introduction

Numerous neurotoxicity studies have been conducted in which hens were exposed to DEF by the intraperitoneal, subcutaneous, inhalation, oral, or dermal route (Tables 12 and 13). Due to the large number of studies, most of these studies will only be discussed briefly. Most of these studies were available as published reports and did not follow the standard protocol recommended in the FIFRA guidelines. One subchronic dermal neurotoxicity study did meet FIFRA guidelines.

B. Animal Studies

1. Parenteral Studies

Casida and coworkers (1963) first reported evidence of OPIDN when chickens developed ataxia 10-14 days after 7-10 daily intraperitoneal injections of DEF at 100 mg/kg/day with and without atropine protection. A similar study conducted by Baron and Johnson (1964) reported muscle weakness, ataxia, paralysis, and degenerative lesions in the sciatic nerve and spinal cord in hens after 3-15 intraperitoneal injections of DEF at 50 and 100 mg/kg/day. Johnson (1970 a&b) also reported evidence of OPIDN in hens after a single subcutaneous injection of DEF at approximately 1,000 mg/kg with an onset around day 8. NTE activity, measured in the brain of two hens 17 and 24 hours after dosing, was reduced to 23% of the control activity.

2. Inhalation Studies

Three inhalation studies were conducted in hens with the exposure ranging from a single 4-hr exposure to daily 6-hr exposures, 5 days/week for 3 weeks (Thyssen and Schilde, 1976a; Thyssen and Schilde, 1978a). Evidence of OPIDN (ataxia, paralysis and nerve degeneration) was observed in all 3 studies. Compared to cholinergic signs, the development of OPIDN appeared to be especially sensitive to repeated inhalation exposure. With a single inhalation exposure, the acute LOAEL for OPIDN was 4-fold higher (878 mg/m^3 or 174 mg/kg[9]) than for cholinergic signs (391 mg/m^3 or 43 mg/kg). However, with 5 consecutive inhalation exposures, the LOAEL for OPIDN was nearly 2-fold lower (145 mg/m^3 or 16 mg/kg/day) than for

[9] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a chicken was assumed to be 0.11 m^3/kg/4 hrs (Dejours *et al.*, 1970). A 100% respiratory retention and absorption was assumed.

Table 12. Acute Neurotoxicity Studies for DEF with Hens

Dosage	Hens/Dose	Effect	NOAEL (mg/kg)	LOAEL (mg/kg)	Ref.[a]
		Inhalation[b]			
391, 878, 1585 mg/m^3, single 4-hr exposure	5	Leg weakness, drowsiness, inactivity, breathing disorders	---	43	1
		Ataxia and paralysis (onset day 15), degeneration of sciatic nerve	97	174	
62, 145 or 246 mg/m^3, 4-hrs/day for 5 days	10	Ataxia (onset day 16-18) (paralysis and nerve degeneration at 27 mg/kg)	6.8	16	1
		Leg weakness, drowsiness, inactivity, breathing disorders	16	27	
		Subcutaneous			
200 or 1,060 mg/kg	2-3	Ataxia, paralysis (onset day 8)	200	1060	2
220 or 1,100 mg/kg	2	Ataxia (onset not reported)	220	1010	3
		Oral			
0, 50 - 500 mg/kg, gavage	10	Death and unspecified toxic effects	50	100	4
0, 50 - 1,000 mg/kg, one capsule	3	"Late acute" effects (onset day 2-14) and ataxia (onset day 4)	50	100	5
0, 100 - 1,000 mg/kg, one capsule	5	Ataxia (onset not reported), peripheral demyelination (1 hen)	---	100	6
		Dermal			
0.5, 1 or 2 ml/kg, dorsal skin	5	Impaired general health, ataxia and paralysis (onset week 2-3)	~500	~1000	7
0, 400 or 1,000 mg/kg, comb	3	Brain ChE inhibition (74% of controls), ataxia (onset day 6-11) (nerve degeneration at 1,000 mg/kg)	---	400	5
100 - 1,000 mg/kg, neck	5	Ataxia and paralysis (onset day 9-10)	100	250	8
		Unspecified mild cholinergic signs	250	500	
0, 100 - 1,000 mg/kg, back of neck	5	Unspecified mild cholinergic signs, ataxia and paralysis (onset not reported)	100	250	6

a References: 1. Thyssen and Schilde, 1976a; 2. Johnson, 1970a; 3. Johnson, 1970b; 4. Thyssen, 1976; 5. Abou-Donia *et al.*, 1979a; 6. Abou-Donia *et al.*, 1984; 7. Thyssen and Schilde, 1976b; 8. Abdo *et al.*, 1983a.

b Air concentrations were converted to mg/kg by assuming 100% respiratory retention and absorption, and a respiratory rate of 0.11 m^3/kg/4 hrs (Dejours *et al.*, 1970).

Table 13. Subchronic Neurotoxicity Studies for DEF in Hens

Dosage	Hens/Dose	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Ref.[a]
		Inhalation[b]			
8, 21 or 84 mg/m^3, 6 hr/day, 5 day/wk, 3 wks	10	Decreased preening, lethargy, ataxia and paralysis (onset week 4), degeneration of sciatic nerve (1 hen)	3.6	14.3	1
		Intraperitoneal			
100 mg/kg/day, 7 or 10 days	NR	Ataxia (onset day 10-14)	---	100	2
50 or 100 mg/kg/day, 3 to 15 days	5-28	Muscle weakness, ataxia, paralysis, nerve degeneration	---	50	3
		Oral			
50, 100, 150 mg/kg/day, 4-15 days	1-7	Unspecified degenerative lesions in spinal cord and sciatic nerve (1 hen)	100	150	3
0, 100, 250, 500 ppm, 30 days, diet	6	Focal liquefication of brain	34[c]	87	4
0, 25 - 400 ppm, 30 days, diet	10	Reduced food consumption, perivascular CNS[d] & PNS inflammation	6.1	10.9	5
0, 0.1 - 80 mg/kg/day, capsule, 90 days	5	Ataxia (onset day 30) "Late acute" effects (onset day 2-5), paralysis (onset day 19-30) and nerve degeneration	0.1 10	0.5 20	6
3 - 40 mg/kg/day capsule, 91-97 days	3-4	Death, ataxia and paralysis (onset day 10-26)	5-6	38-40	7
		Dermal			
0, 0.01 - 1 ml/kg/day, 6 hr/day, 5 days/wk, 3 wks, axilla	8	Ataxia, paralysis (onset week 3) Unspecified cholinergic signs	~30 ~100	~100 ~300	8
0, 20, 40 mg/kg/day, 90 days, comb	3	Ataxia (onset day 8-22)	---	20	6
6 - 16 mg/kg/day 91-101 days, comb	3	Ataxia (onset day 76-100); skin: thickening of keratin and epidermis, collagen deposition, inflammation	---	6-8	7
0,2.6,11,42 mg/kg/day, 5 day/wk, 13 wks, comb	12	Axonal degeneration	2.6	11	9*

a References: 1. Thyssen and Schilde, 1978a; 2. Casida *et al.*, 1963; 3. Baron and Johnson, 1964; 4. Harris, 1965; 5. Thyssen *et al.*, 1977; 6. Abou-Donia *et al.*, 1979b; 7. Hansen *et al.*, 1982; 8. Thyssen and Schilde, 1978b; 9. Sheets, 1991b.

b Air concentrations were converted to mg/kg by assuming 100% respiratory retention and absorption, and a respiratory rate of 0.17 m^3/kg/6 hrs (Dejours *et al.*, 1970).

c Using the mean food consumption for each group from the study and assuming a body weight of 2 kg.

d CNS = central nervous system; PNS = peripheral nervous system

* Acceptable study based on FIFRA guidelines

cholinergic signs (246 mg/m^3 or 27 mg/kg/day). The subchronic NOAEL for OPIDN was 21 mg/m^3 (3.6 mg/kg/day)[10]. None of these studies met FIFRA guidelines.

3. Oral Studies

Several of the initial oral studies for DEF suggested that OPIDN was not easily produced by this route. Baron and Johnson (1964) did not observe any evidence of OPIDN in hens when DEF was administered by oral gavage at 50-150 mg/kg for 4-15 days with the possible exception of one hen. In a 30-day feeding study, there was equivocal histological evidence of OPIDN (demyelination of the spinal cord at 100 and 250 ppm only; focal liquefication of the brain at 250 and 500 ppm) in 1 of 6 hens per dose when fed DEF at 100, 250 or 500 ppm (Harris, 1965). Thyssen (1976) found no clinical or histological evidence of OPIDN when hens were administered DEF by oral gavage at 300 mg/kg twice with a 21-day interval between each dose. Equivocal histological evidence of OPIDN (perivascular CNS and PNS inflammation) was also seen in another 30-day study in which hens were fed DEF at 0, 25, 50, 100, 200 or 400 ppm (Thyssen *et al.*, 1977).

One explanation for the reduced incidence of OPIDN in hens administered DEF by the oral route may be the hydrolysis of DEF to nBM in the gastrointestinal tract. Abou-Donia *et al.* (1979a) first reported "late acute" effects in hens administered a single capsule containing DEF at 100 mg/kg or higher. The hens exhibited leg weakness, unsteadiness and a yellowish watery liquid around the mouth by the second day after dosing. Their condition progressively worsened with malaise, general muscle weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors and loss of breath which were not responsive to atropine therapy. Just prior to death, their combs became dark and droopy. These late acute effects were distinguished from those associated with OPIDN in that the onset of death was earlier (2-14 days after dosing) and no histological lesions were found in the sciatic nerve. nBM was identified by mass spectrometry in the excreta of hens administered DEF orally. These investigators tested the possibility that these effects were due to nBM by administering hens a single capsule containing nBM (98%) at 100, 400 or 1,000 mg/kg. The hens at 400 and 1,000 mg/kg developed clinical signs similar to the late acute effects observed when DEF was administered orally; however, the onset of signs was earlier (6-12 hrs after administration). No clinical signs were observed in hens at 100 mg/kg. No degenerative changes in the sciatic nerve were present in any of the hens treated with nBM.

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[10] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a chicken was assumed to be 0.17 m^3/kg/6 hrs (Dejours *et al.*, 1970). A 100% respiratory retention and absorption was assumed.

Furthermore, there was a slight increase or no change in brain and plasma ChE activity of hens exposed to nBM. The half-life of nBM was estimated to be 8 days based on the plasma levels.

A mechanism of action for nBM toxicity was proposed by Abdo *et al.* (1983b) who found an increase in Heinz bodies and extensive erythrocyte deformation and lysis 24 to 48 hrs after hens were given nBM in capsules at 500 mg/kg. Methemoglobin levels were significantly higher in the treated birds while the hemoglobin concentration, packed cell volume, erythrocytes and glucose-6-phosphate dehydrogenase (G-6-PD) activity were significantly lower. The time course for disappearance of hematological changes and late acute effects was similar and the investigators suggested that the inhibition of G-6-PD by nBM led to the hematological changes. This enzyme is required to regenerate nicotinamide adenine dinucleotide phosphate (NADPH) which is needed for the reduction of glutathione. Decreased levels of reduced glutathione resulted in the denaturation of hemoglobin (i.e., formation of methemoglobin and Heinz bodies), coagulation of surface proteins on erythrocytes leading to deformation, and eventual cell lysis. The investigators concluded the late acute effects observed after oral administration of DEF were directly related to the hematological changes.

Evidence of OPIDN was observed in four other oral neurotoxicity studies with hens (Abou-Donia *et al.*, 1979a&b; Abou-Donia *et al.*, 1984; Hansen *et al.*, 1982). An acute NOAEL of 50 mg/kg/day was established for OPIDN in one study (Abou-Donia *et al.*, 1979a). A significantly lower NOAEL of 0.1 mg/kg/day was observed in a oral subchronic study with hens based on mild ataxia (Abou-Donia *et al.*, 1979b). However, there was limited histological evidence of delayed neuropathy with oral exposure even at high doses (Table 14). Unequivocal histological lesions in the spinal cord or peripheral nerve were not observed in this study until the dose was increased to 20 mg/kg. At 80 mg/kg/day, 1 out of 5 hens had unequivocal lesions and 2 out of 5 hens had equivocal lesions in the spinal cord indicative of OPIDN. The equivocal lesions were lesions Abou-Donia *et al*. (1979b) suggested could be early signs of delayed neuropathy, but because they were occasionally observed in controls he could not be certain. However, there was no dose-response relationship in the incidence of the equivocal lesions with oral exposure. More likely these lesions were age-related because the birds were relatively old (19 months). Abou-Donia *et al*. (1979b) did not report the incidence of the histological lesions at 0.1 mg/kg/day or in the controls, making interpretation of the equivocal lesions difficult. Hens receiving 20-80 mg/kg/day orally, developed severe ataxia, some became paralyzed and nearly all died from late acute effects within the first few weeks of exposure. Abou-Donia *et al*. (1979b) suggested that more histological lesions would have been seen with oral exposure if the

Table 14. Incidence of Histological Lesions in Spinal Cord or Peripheral Nerve[a]

Dose mg/kg/day	Oral[b]			Dermal[c]		
	Positive	Equivocal	Negative	Positive	Equivocal	Negative
80	1	2	2	—	—	—
40	0	1	4	3	0	0
20	1	0	4	0	1	2
10	0	1	4	—	—	—
5	0	1	4	—	—	—
2.5	0	0	5	—	—	—
1	0	2	3	—	—	—
0.5	0	2	3	—	—	—

[a] Abou-Donia *et al.*, 1979b

[b] Five hens exposed by the oral route per dose level

[c] Three hens exposed by the dermal route per dose level

hens had lived longer. However, if there was enough nBM to kill these hens, there probably was significantly less DEF available to produce delayed neuropathy. Moreover, the nBM was probably responsible for the ataxia and paralysis with oral exposure because of the limited evidence of delayed neuropathy even at lethal doses. Abou-Donia *et al*. (1979b) and Fairchild and Stokinger (1958) reported that nBM caused muscle weakness, incoordination, paralysis, CNS depression and cyanosis all of which could affect gait. NTE activity was not measured in the Abou-Donia *et al.* (1979b) study which would have helped in the interpretation of the clinical and histopathological findings. A LOAEL of 20 mg/kg/day for OPIDN would be more consistent with the LOAEL reported for a similar oral subchronic neurotoxicity study in which death, paralysis, and nerve degeneration were observed in hens at approximately 40 mg/kg/day (Hansen *et al.*, 1982). None of the oral neurotoxicity studies for DEF met FIFRA guidelines because of inadequate exposure duration, inadequate number of hens per group, age of hens, no analysis of test article or dosing material, inadequate or no histopathology data or no positive controls.

4. Dermal Studies

Evidence of OPIDN was observed in nearly all of the acute dermal neurotoxicity studies in hens (Thyssen and Schilde, 1976b; Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984; Abdo *et*

al., 1983a; Thyssen and Schilde, 1978b; Abou-Donia *et al.*, 1979b; Hansen *et al.*, 1982; Sheets, 1991b). The lowest acute NOAEL for OPIDN by the dermal route was 100 mg/kg (Abdo *et al.*, 1983a; Abou-Donia *et al.*, 1984). None of the acute dermal neurotoxicity studies met FIFRA guidelines.

There was evidence of OPIDN in a number of subchronic dermal neurotoxicity studies in hens (Thyssen and Schilde, 1978b; Abou-Donia *et al*, 1979b; Hansen *et al.*, 1982; Sheets, 1991b). It is interesting to compare the findings with oral and dermal exposure from the Abou-Donia *et al.* (1979b) study at 40 mg/kg/day (Table 14). With oral exposure, 4 out of 5 hens had no lesions and 1 out of 5 hens had an equivocal lesion in the spinal cord suggestive of OPIDN. By contrast, with dermal exposure unequivocal lesions of OPIDN were observed in the spinal cord of all 3 hens. While all the hens administered DEF at 40 mg/kg/day by the oral route died within the first few weeks of exposure, none of the hens administered DEF at this same dose level by the dermal route died despite developing clear evidence of delayed neuropathy. A NOAEL could not be established for delayed neuropathy with dermal exposure because animals at the lowest dose developed ataxia which, unlike with oral exposure, was probably related to OPIDN since significantly less nBM would likely be formed with this route of exposure.

Only the subchronic dermal neurotoxicity study conducted by Sheets (1991b) was acceptable to DPR toxicologists based on the FIFRA guidelines. In this study, 12 white leghorn hens/group were administered DEF (97.7%) topically to the comb at 0, 2.6, 11, and 42 mg/kg/day for 5 days/week for 13 weeks. Whole blood cholinesterase activity was significantly reduced at 2.6 mg/kg/day (53% of controls), 11 mg/kg/day (43% of controls), and 42 mg/kg/day (43% of controls). Decreased motor activity and ataxia were observed in all hens at 42 mg/kg/day with an onset between days 12 and 39. There was a high background rate for axonal degeneration probably due to the age of the birds (17 months) which were older than recommended by FIFRA guidelines (8-14 months), thus making interpretation of the histological findings difficult. The axonal degeneration was identical to that encountered in older hens that have had vaccines or other exogenous viral exposure, such as Marek's disease. There was a statistically significant increase in the severity of the axonal degeneration at 42 mg/kg/day. Although not statistically significant, the slight increase in severity and incidence of axonal degeneration at 11 mg/kg/day was considered toxicologically significant by DPR toxicologists. There were only two instances of mild ataxia on days 71 and 80 in 1 of 12 hens at 11 mg/kg/day, suggesting that most of axonal degeneration was age-related. DPR toxicologists made the health protective assumption that the axonal degeneration was treatment-related and set the NOAEL at 2.6 mg/kg/day. The LOAEL in this study is consistent with the LOAELs established in two other subchronic dermal neurotoxicity

studies (Abou-Donia *et al.*, 1979b; Hansen *et al.*, 1982); however, the NOAEL was the lowest subchronic NOAEL observed for OPIDN by the dermal route.

A study was conducted to evaluate the neurotoxic effects of DEF in hens from normal field use. Scaleless hens were exposed to varying levels of DEF over a 7-hour period based on their proximity to a cotton field that was sprayed with DEF by a rig (Wilson *et al.*, 1980). Dermal exposure was estimated by measuring residues on mylar sheets placed next to the hens. The estimated dermal exposure ranged from 0.0092 $\mu g/cm^2$ in unsprayed rows of cotton to 47.8 $\mu g/cm^2$ on the rig near the hens exposed for one day. The dermal exposure for hens exposed daily in treated rows for a week was estimated to be 108 $\mu g/cm^2$. Air concentrations of DEF were also measured and ranged from 0.111 mg/m^3 in untreated rows to 13.8 mg/m^3 near the rig. None of the hens exhibited ataxia or other signs of OPIDN.

C. Human Studies

Kilgore *et al.* (1984) conducted a study with pesticide workers before and after a 7-week exposure period to DEF in which medical examinations and neuro-psychological tests were performed. No significant effects were found including cholinesterase inhibition. Another worker exposure study was conducted by Lotti *et al.* (1983) in which pesticide workers were monitored before and after the normal use season. No differences were detected between pre- and post-exposure electromyographs and nerve conduction tests. The whole blood and plasma ChE levels were all within 25% of pre-exposure levels. However, the lymphocyte NTE activity was reduced to between 40 and 60% of pre-exposure levels between days 25 and 30 of exposure. Neither of these studies provided sufficient information to accurately estimate total DEF exposure.

D. Conclusions

Delayed neuropathy was observed in acute and subchronic studies in which hens were exposed by the inhalation, oral, and dermal route. In addition, cholinergic signs and other effects described as "late acute effects" were seen; however, the late acute effects were only observed with oral exposure in hens. The late acute effects were attributed to the hydrolysis of DEF to nBM which was found in the excreta of hens administered DEF orally. In subsequent studies, it was shown in hens that nBM causes erythrocyte deformation and lysis through the inhibition of glucose-6-phosphate dehydrogenase. With acute oral exposure in hens, the OPIDN occurred at approximately the same dose levels or higher than the cholinergic and late acute effects. However, the distinction between the cholinergic, late acute, and delayed neurotoxic effects was blurred in some studies because some effects such as leg weakness and unsteadiness were

common to all syndromes and could only be separated based on their time of onset. There is also some uncertainty about the dose levels at which cholinergic signs occurred because many of the studies provided insufficient information about the incidence of cholinergic effects to accurately determine a NOAEL.

The lowest NOAEL with acute inhalation exposure to DEF was less than 43 mg/kg based on leg weakness, drowsiness, inactivity, and breathing disorders (Thyssen and Schilde, 1976a). With acute oral exposure, the lowest NOAEL was 50 mg/kg based on death, unspecified toxic signs, late acute effects, and ataxia (Thyssen, 1976; Abou-Donia *et al.*, 1979a). The lowest acute dermal NOAEL was 100 mg/kg based on unspecified mild cholinergic signs, ataxia and paralysis (Abou-Donia *et al.*, 1984; Abdo *et al.*, 1983a). The lowest NOAEL with subchronic inhalation exposure to DEF was 3.6 mg/kg/day based on decreased preening, lethargy, ataxia, paralysis, and nerve degeneration (Thyssen and Schilde, 1978a). The lowest subchronic oral NOAEL was 0.1 mg/kg/day based on mild ataxia (Abou-Donia *et al.*, 1979b). However, it was uncertain if the mild ataxia observed at 0.5 mg/kg/day was due DEF or the degradation product, nBM, which can also cause incoordination and muscle weakness. The lack of unequivocal histological lesions in the spinal cord or sciatic nerve until 20 mg/kg suggests that the ataxia at lower doses was probably due to nBM. The more pronounced histological lesions in hens exposed dermally to DEF at 40 mg/kg/day compared to those observed in hens exposed orally at the same dose level, also suggests that the ataxia at the lower doses is due to nBM rather than DEF. If DEF is degrading to nBM in the gut as Abou-Donia and coworkers have suggested, the incidence or severity of OPIDN should be less by the oral route. With subchronic dermal exposure to DEF, the lowest NOAEL was 2.6 mg/kg/day based on axonal degeneration (Sheets, 1991b).

XII. RISK ANALYSIS

A. Introduction

The risk assessment process consists of four basic elements: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and characterizes the relationship between the amount of exposure (or dose) and the severity or probability of a toxic effect. The amount of exposure which will not likely result in an observable or significant adverse health effect is estimated. In this risk assessment, the exposure assessment includes an estimation of the potential exposure through ambient air on an acute, subchronic, and chronic basis. Risk characterization then extrapolates the toxic effects observed in the laboratory studies, in which animals are exposed to high dosages of pesticide, to potential human exposures at the low dosages of pesticide residues in ambient air.

B. Hazard Identification

The dose-response assessment has been integrated into the hazard identification section in this risk assessment.

1. Acute Toxicity

a. DEF

The effects observed in experimental animals after acute exposure to DEF are summarized in Table 15. In addition to the effects observed in the LD_{50}/LC_{50} studies and the acute neurotoxicity studies, some findings observed in the 90-day inhalation, 21-day dermal, and developmental toxicity studies were also considered as acute effects. These include signs observed during the first few days of exposure and any fetal effects in the developmental toxicity studies, assuming they were the result of a single exposure. The clinical signs observed after acute exposure to DEF were primarily neurological. Cholinergic signs were seen in most laboratory animals after acute exposure to DEF by various routes. Hypothermia was observed in rats, mice and guinea pigs when DEF was administered by the oral, intraperitoneal and intravenous route (Ray, 1980; Ray and Cunningham, 1985). The investigators suggested a selective action on a central thermogenic control process may be involved. Other research

Table 15. Acute Adverse Effects of DEF and Their Respective NOAELs and LOAELs

Species	Exposure	Effect	NOAEL (mg/kg)	LOAEL (mg/kg)	Ref.[a]
		Inhalation[b]			
Rat	Single, 4-hr, nose only	Death, cholinergic signs, red turbinates, firm zones in lungs	---	254	1*
Rat	Single, 4-hr	Decreased preening, lethargy	12.3	20.8	2
Rat	**13 weeks, 6 hr/day, 5 day/wk**	**Reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, increased startle response (onset days 1-3)**	**2.9**	**14.3**	**3***
		Blood ChE[c] inhibition (54-75%) (week 1)	**0.6**	**2.9**	
Hen	Single, 4-hr	Leg weakness, drowsiness, inactivity, breathing disorders	---	43	4
	5 Days, 4-hr	Ataxia	6.8	16	
		Intraperitoneal			
Rat	Single, injection	Hypothermia	---	20	5
		Oral			
Rat	Single, gavage	Cholinergic signs	---	192	6*
Rat[d]	9 Days, gavage	Salivation (onset day 3)	7	28	7*
Hen	Single, gavage	Death, unspecified toxic effects	50	100	8
Hen	Single, capsule	"Late acute" effects, ataxia	50	100	9
Hen	Single, capsule	Ataxia, peripheral demyelination	---	100	10
		Subcutaneous			
Hen	Single, injection	Ataxia, paralysis	200	1060	11
Hen	Single, injection	Ataxia	220	1010	12
		Dermal			
Rabbit	Single, 24-hr	Cholinergic signs, erythema	---	500	13*
Rabbit	3 weeks, 6 hr/day, 5 days/wk	Muscle fasciculations (onset day 2)	11	29	14*
Hen	Single, 24-hr	Impaired general health, ataxia, paralysis	~500	~1000	15
Hen	Single	Brain ChE inhibition, ataxia (nerve degeneration at 1,000 mg/kg)	---	400	9
Hen	Single	Ataxia, paralysis	100	250	16
Hen	Single	Ataxia, paralysis, cholinergic signs	100	250	10

[a] References: 1. Warren, 1990; 2. Thyssen, 1978a; 3. Pauluhn, 1992; 4. Thyssen and Schilde, 1976a; 5. Ray, 1980; 6. Sheets, 1991a; 7. Kowalski *et al.*, 1986; 8. Thyssen, 1976; 9. Abou-Donia *et al.*, 1979a; 10. Abou-Donia *et al.*, 1984; 11. Johnson, 1970a; 12. Johnson, 1970b; 13. Sheets and Phillips, 1991; 14. Sheets *et al.*, 1991; 15. Thyssen and Schilde, 1976b; 16. Abdo *et al.*, 1983a.

[b] Estimated assuming 100% respiratory retention and absorption, and a respiratory rate of 0.16 and 0.11 m^3/kg/4 hrs for a rat and hen, respectively.

[c] ChE = cholinesterase. Inhibition is expressed as percent of control activity.

[d] Developmental toxicity study: All fetal effects were considered acute effects; however, only maternal effects observed within the first few days of exposure were considered acute effects.

* Acceptable study based on FIFRA guidelines.

indicates that the hypothermia associated with organophosphates is due to central AChE inhibition because hypothermia is antagonized by centrally active antiChE drugs, such as atropine, but not by peripherally active antiChE drugs, such as 2-PAM (Kenley *et al.*, 1982). Organophosphate-induced delayed neuropathy (OPIDN) was observed in hens in the form of ataxia, paralysis, and nerve degeneration approximately 2-3 weeks after acute exposure to DEF by inhalation, oral or dermal routes. There was no evidence of DEF-induced delayed neuropathy in other species tested; however, rodents generally are less susceptible to OPIDN (Abou-Donia, 1981; Somkuti *et al.*, 1988; De Bleeker *et al.*, 1992). Sensitivity in rodents appears to vary depending on strain and age (Padilla and Veronesi, 1988; Veronesi *et al.*, 1991; Moretto *et al.*, 10992; Inui *et al.*, 1993).

Other effects described as "late acute" effects were also seen in hens; however, the late acute effects were only observed with oral exposure (Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984). Abou-Donia and coworkers attributed these late acute effects to nBM which was found in the excreta of hens after oral exposure, presumably from the hydrolysis of DEF in the gut. In subsequent studies, it was shown that in hens nBM causes erythrocyte deformation and lysis through the inhibition of G-6-PD (Abdo *et al.*, 1983b). Clinical signs similar to the late acute effects in hens have not been described in other species administered DEF, but changes in erythrocyte morphology were seen in rabbits administered DEF (route not indicated) at 242 mg/kg (Mirakhmedov *et al.*, 1989). In addition, mice, rats, and dogs had reductions in erythrocyte counts, hematocrits and hemoglobin after long-term exposure to DEF in the diet (Hayes, 1989; Christenson, 1991; Christenson, 1992). Similar hematological changes were also seen at the termination of a 90-day inhalation study in rats, presumably from either degradation of DEF in the chamber or normal tissue metabolism of DEF (Pauluhn, 1992).

OPIDN occurred at approximately the same dose levels or higher than the cholinergic and late acute effects occurred in hens. The distinction between the cholinergic, late acute and OPIDN was unclear in some hen studies because several effects such as leg weakness and unsteadiness are common to all 3 syndromes and could only be separated based on their time of onset. There is also some uncertainty about the dose levels at which cholinergic signs occurred because many of the hen studies provided insufficient information about the incidence of cholinergic effects to accurately determine a NOAEL. In mammals, cholinergic signs were the primary effects observed after acute exposure to DEF. In an acceptable acute inhalation LC_{50} study, death, unthriftiness, hypoactivity, urine stains, nasal discharge, red eye discharge, lacrimation, ataxia, tremors, excitability, vocalization, dyspnea, red turbinates, and firm zones in the lungs were observed in rats at 1,590 mg/m^3 (254 mg/kg) with a single, 4-hour exposure (Warren, 1990). However, like most LD_{50}/LC_{50} studies the dose levels were too high in this study

to establish a NOAEL. NOAELs were observed in two other acute inhalation studies. In an another LC_{50} study, a NOAEL was established at 77 mg/m^3 (12.3 mg/kg) with a single, 4-hour inhalation exposure based on decreased preening and lethargy in rats exposed at 130 mg/m^3 (Thyssen, 1978a). A slightly lower NOAEL was observed in an inhalation neurotoxicity study at 62 mg/m^3 (6.8 mg/kg) based on ataxia in hens exposed for 5 consecutive days at 145 mg/m^3 (Thyssen and Schilde, 1976a). The NOAELs from these studies were not used because both of these studies had major deficiencies, including no analysis of particle size, no summary of clinical signs by dose group, and no gross necropsy.

An acute NOAEL of 12.2 mg/m^3 (2.9 mg/kg/day) was established in a 90-day inhalation study in rats based on reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, and increased startle response that were observed within the first 3 days of dosing at 59.5 mg/m^3 (14.3 mg/kg/day) (Pauluhn, 1992). This study also met FIFRA guidelines. Although most of these signs are not clearly cholinergic in origin, they may have been due to a localized response in the lungs. Findings in other studies suggest that there was irritation to the respiratory tract, including red nasal turbinates and "firm zones" in the lungs in an inhalation LC_{50} study (Warren, 1990) and inflammation in the lungs in a 3-week inhalation study (Thyssen, 1978b). The early signs in the 90-day inhalation study were also consistent with the signs observed in the inhalation LC_{50} studies. Most of these signs were not observed until more than one dose was administered; therefore, it is possible that the NOAEL after a single day of exposure would be higher. However, the general public is more likely to be exposed to DEF in ambient air for more than one day. Therefore, the 90-day inhalation study in rats was selected by DPR toxicologists as the definitive study for evaluating acute exposure to DEF in ambient air with a critical NOAEL of 12.2 mg/m^3 (2.9 mg/kg/day) based on clinical signs .

In general, DPR toxicologists do not consider plasma or erythrocyte ChE inhibition in the absence of clinical signs or symptoms to be an adverse effect because the ChEs in blood have no known physiological function. However, after reviewing the Health Assessment document for the Evaluation of DEF as TAC, the SRP for TACs concluded that blood ChE inhibition should be considered an adverse effect because of the possible role of plasma ChE in the metabolism of various drugs, such as succinylcholine. Consequently, NOAELs were identified for blood ChE inhibition in the definitive study along with the critical NOAELs that DPR toxicologists had previously selected to evaluate exposure to DEF in ambient air. The acute NOAEL for blood ChE inhibition in the 90-day inhalation study for DEF was 2.4 mg/m^3 (0.6 mg/kg/day) based on a significant reduction in ChE activity in the plasma (F: 54% of controls) and erythrocytes (M: 64%; F: 75% of controls) at 12.2 mg/m^3 during the first week of exposure.

b. n-Butyl Mercaptan

Only limited toxicity data were available for nBM. Effects observed in a battery of acute toxicity tests (intraperitoneal LD_{50}, oral LD_{50}, inhalation LD_{50}, ocular irritation) were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma (Fairchild and Stokinger, 1958). Other effects included restlessness, increased respiration, diarrhea (oral exposure), sneezing (inhalation exposure), and ocular irritation. Liver damage (lymphatic infiltration and necrotic foci with small hemorrhages) and kidney damage (cloudy swelling of the tubules and hyaline casts in the lumina) were observed with all routes of exposure. With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lungs were seen. There was insufficient information available in the published report by Fairchild and Stokinger (1958) to establish a NOAEL by any of the routes tested. An inhalation developmental toxicity study was available in which mice and rats were exposed to vapors of nBM for 6 hrs/day on gestation days 6-16 and 6-19, respectively (Thomas *et al.*, 1987). No maternal or developmental effects were seen in rats. The NOAEL for the maternal and developmental effects in mice was 10 ppm (17 mg/kg/day) based on increased mortalities, reduced body weight gain, and clinical signs in the dams (unkempt appearance, lethargy, red/brown perianal staining), increased post-implantation losses, and fetal malformations.

As mentioned earlier, the "late acute" effects seen in hens with oral exposure were attributed to nBM which is probably formed in the gut from the hydrolysis of DEF (Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984). These investigators tested this theory by administering nBM to hens and found they developed signs similar to those described as late acute effects (malaise, leg or general weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors, loss of breath, and dark and droopy comb just prior to death), except the onset of signs was earlier (6-12 hrs after administration). Hens administered nBM did not respond to atropine therapy, did not have any inhibition of brain or plasma ChE activity, and did not develop degenerative changes in peripheral nerves. A NOAEL of 100 mg/kg was observed based on clinical signs in hens administered nBM. Abdo *et al*. (1983b) observed erythrocyte deformation and lysis in hens 24-48 hrs after administering nBM at 500 mg/kg. Methemoglobin levels were elevated while erythrocyte counts, hematocrit, hemoglobin levels and G-6-PD activity were reduced. Because the time course of the hematological changes and the late acute effects were similar, the investigators proposed that the inhibition of G-6-PD was responsible for the hematological changes. G-6-PD is involved in the regeneration of NADPH which in turn is needed for the reduction of glutathione. A reduction in glutathione levels could lead to the formation of methemoglobin and Heinz bodies, coagulation of surface proteins on erythrocytes,

leading to deformation and eventual cell lysis. Since only one dose was administered in this study, a NOAEL was not established for the hematological changes.

Residents of agricultural communities in cotton-growing regions have complained of eye and throat irritation, rhinitis, wheezing, coughing, shortness of breath, nausea and diarrhea during the time of cotton defoliation with DEF (Maddy and Peoples, 1977; Scarborough, 1989). Based on the acute effects seen in animals exposed to nBM, it appears that nBM may be responsible for the ocular and respiratory irritation. Some of these complaints may also be due to the strong skunk-like odor of nBM. The odor threshold of nBM in humans is between 0.01 and 1.0 ppb (Santodonato *et al.,* 1985). Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms including exacerbating an underlying medical condition, innate odor aversion, odor-related aversive conditioning, stress-induced illness, and possible innate pheromonal reaction (Shusterman, 1992). Ames and Stratton (1991) analyzed health effects reported by residents living near a potato field that had been treated with ethoprop which breaks down to n-propyl mercaptan. They found that symptoms more closely correlated with odor perception than with distance from the potato field.

2. Subchronic Toxicity

The effects from subchronic exposure to DEF in experimental animals are summarized in Table 16. Included in this summary are some maternal effects from developmental toxicity studies that were not evident until after the first few days of exposure and all effects observed in reproductive toxicity studies. Not included in this table were two neurotoxicity studies in which hens were given daily intraperitoneal injections at 50 and 100 mg/kg for 5 to 15 days (Casida *et al.*, 1963; Baron and Johnson, 1964). Baron and Johnson (1964) also observed OPIDN in hens administered DEF by oral gavage at 50-150 mg/kg/day for 5 to 15 days. These two studies were not used because NOAELs were not established and they had major deficiencies including inadequate number of animals and inconsistent exposure periods within and between dose levels. Also, not included was a 3-month feeding study in rats and dogs (Root and Doull, 1966). A NOAEL of 5 ppm (0.25 and 0.125 mg/kg/day for rats and dogs, respectively) was reported for both species, but the effects seen at the LOAEL were not reported. This study had other major

Table 16. Subchronic Adverse Effects of DEF and Their Respective NOAELs and LOAELs

Species	Exposure	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Ref.[a]
		Inhalation[b]			
Rat	6 hr/day, 5 day/wk, 3 weeks	Brain ChE[c] inhibition (73%), decreased preening, lethargy, inflammation in lung	1.7	7.7	1
Rat	6 hr/day, 5 day/wk, 2 weeks	Cholinergic signs, brain ChE inhibition (61%)	3.2	15	2
Rat	**6 hr/day, 5 day/wk, 13 weeks**	**Clinical signs, hematological changes, brain ChE inhibition (60%), impaired retinal function, pale retinal fundus, fatty droplets in adrenals, increased adrenal wts.**	**2.9**	**14.3**	**3***
		Blood ChE inhibition (35-60%)	**0.6**	**2.9**	
Hen	6 hr/day, 5 day/wk, 3 weeks	Decreased preening, lethargy, ataxia, paralysis, nerve degeneration	3.6	14.3	4
		Oral			
Mouse	8 weeks, feed	Brain ChE inhibition (74%)	40	140	5
Rat[d]	9 days, gavage	Reduced maternal weight gain and maternal brain ChE inhibition (54%)	7	28	6*
Rat[e]	Diet, 2-gen., 10 wk/gen.	**Parental:** Brain ChE inhibition (F: 71%)	0.4	3.0	7*
		Reproductive: Reduced fertility, birth and viability indices, increased gestation length, reduced pup weights, cannibalism of pups and discolored pup livers	3.0	24.1	
Rabbit[d]	12 days, gavage	Reduced maternal weight gain	3	9	8*
Hen	30 days, feed	Focal liquefication of brain	34	87	9
Hen	30 days, feed	Reduced food consumption, neurohistological lesions	6.1	10.9	10
Hen	90 days, capsule	Ataxia	0.1	0.5	11
Hen	91-97 days, capsule	Death, ataxia, paralysis	5-6	38-40	12
		Dermal			
Rabbit	6 hr/day, 5 day/wk, 3 weeks	Muscle fasciculations, brain ChE inhibition (85%), skin lesions	2	11	13*
Hen	6 hr/day, 5 day/wk, 3 weeks	Ataxia, paralysis	~30	~100	14
Hen	90 days	Ataxia	--	20	11
Hen	91-101 days	Ataxia, dermal irritation	--	6-8	12
Hen	13 weeks, 5 day/wk	Axonal degeneration	2.6	11	15*

a References: 1. Thyssen, 1978b; 2. Pauluhn, 1991; 3. Pauluhn, 1992; 4. Thyssen and Schilde, 1978a; 5. Hayes, 1985; 6. Kowalski *et al.*, 1986; 7. Eigenberg, 1991a; 8. Clemens *et al.*, 1987; 9. Harris, 1965; 10. Thyssen *et al.*, 1977; 11. Abou-Donia *et al.*, 1979b; 12. Hansen *et al.*, 1982; 13. Sheets *et al.*, 1991; 14. Thyssen and Schilde, 1978b; 15. Sheets, 1991b.

b Estimated assuming 100% respiratory retention and absorption, and a respiratory rate of 0.24 and 0.17 m^3/kg/6 hrs for a rat and hen, respectively.

c ChE = cholinesterase. Inhibition is expressed as percent of control activity.

d Developmental toxicity study: Only maternal effects observed after the first few days of exposure were included.

e Reproductive toxicity study

* Acceptable study based on FIFRA guidelines

deficiencies such as no summary of body weights, food consumption, hematology, clinical chemistry or pathological lesions.

The effects observed with subchronic exposure to DEF are similar to the acute effects, including mild cholinergic signs, OPIDN and hematological changes. Additional effects observed with subchronic exposure included reduced weight gain, reduced food consumption, impaired retinal function, pale retinal fundus, fatty droplets in the adrenal cortex, and increased adrenal weights were observed with subchronic exposure. Several reproductive effects were observed in a 2-generation rat reproductive toxicity study including a reduction in fertility, birth and viability indices, an increase in gestation length, reduced pup weights, cannibalism of pups, and discolored pup livers. The lowest inhalation subchronic NOAEL was 7 mg/m^3 (1.7 mg/kg/day) based on mild cholinergic signs and brain ChE inhibition (73% of control activity) in rats exposed to DEF at 32 mg/m^3 for 6 hrs/day, 5 days/week for 3 weeks (Thyssen, 1978b). However, this study had major deficiencies including no analyses of airflow, particle size or temperature in the chambers during exposure, inadequate exposure duration, and inadequate pathological examination.

Instead, a slightly higher NOAEL of 12.2 mg/m^3 (2.9 mg/kg/day) from a 13-week inhalation study with rats was selected as the critical NOAEL for evaluating the seasonal exposure to DEF in ambient air because the study duration was more similar to the estimated seasonal exposure period for humans of 60 days (see Part B, Exposure Assessment, of the report for DEF as a TAC) and the study met FIFRA guidelines (Pauluhn, 1992). The effects observed at the LOAEL (59.5 mg/m^3 or 14.3 mg/kg/day) included clinical signs (reduced motility, bradypnea, irregular breathing, dyspnea, increased aggressiveness, miosis, exophthalmos, vocalization, piloerection, ungroomed coat, convulsions, blepharospasm, hypothermia), hematological changes, brain ChE inhibition (M&F: 60% of controls), impaired retinal function, pale retinal fundus, fatty droplets in the adrenal cortex, and increased adrenal weights. The subchronic NOAEL for blood ChE inhibition in this study was 2.4 mg/m^3 (0.6 mg/kg/day) based on significant reductions in ChE activity in the plasma (F: 44-60% of controls) and erythrocytes (M: 35-50%; F: 36-52% of controls) at 12.2 mg/m^3 (2.9 mg/kg/day) from weeks 4 through 13 of the study.

Lower NOAELs for DEF were observed with two oral subchronic studies. In an acceptable reproductive toxicity study, a NOAEL of 4 ppm (0.4 mg/kg/day) was established based on brain ChE inhibition (71% of control activity) in adult female rats at 32 ppm (2.4 mg/kg/day) (Eigenberg, 1991a). There were gender-related differences in ChE activity in this study which were most pronounced at the terminal sacrifice. However, gender-related differences in brain ChE activity were not observed in the 2-year feeding study in rats where the reduction in

brain ChE inhibition at the LOAEL, 320 ppm (19 mg/kg/day), was similar in both sexes (M: 40%; F: 32% of controls) (Christenson, 1992). In fact, the NOAEL for brain ChE inhibition in the 2-year feeding study (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day) was comparable to the NOAEL for brain ChE inhibition in males in rat reproductive toxicity study (2.2 mg/kg/day). An increased sensitivity in pregnant females also does not appear to be a likely explanation because the maternal NOAEL for brain ChE inhibition in the rat developmental toxicity study was 7 mg/kg/day (Kowalski *et al.*, 1986). A more likely explanation for these gender-related differences in the reproduction study was the higher compound consumption in females during lactation. During lactation, the average compound consumption for females in both generations was approximately twice as high as their consumption during premating and gestation (0.7, 5.5, and 39.2 mg/kg/day at 4, 32, and 260 ppm, respectively). Because of the route of exposure and the uncertainty regarding the impact of the increased compound consumption in the females during lactation on the brain ChE activity, this study was not selected as the definitive study for evaluating seasonal exposure to DEF in ambient air.

A NOAEL of 0.1 mg/kg/day was reported in a neurotoxicity study in hens in which mild ataxia was observed at 0.5 mg/kg/day when DEF was administered in capsules for 90 days (Abou-Donia *et al.*, 1979b). It is unclear if the ataxia with oral exposure was due to DEF or nBM which can be formed in the gastrointestinal tract of hens from the hydrolysis of DEF (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). There was limited histological evidence of OPIDN with oral exposure even at high doses. By contrast, similar doses of DEF administered by the dermal route produced clear evidence of OPIDN. Hens as 20 mg/kg/day and higher had severe ataxia and paralysis and died within the first few weeks from late effects attributed to nBM. The limited histological evidence of OPIDN with oral exposure, suggests that the ataxia and paralysis is due to nBM rather than DEF. Abou-Donia and others have reported that nBM causes incoordination, muscle weakness, paralysis, CNS depression and cyanosis in rats and/or hens (Fairchild and Stokinger, 1958; Abou-Donia *et al.*, 1979a). If sufficient amounts of DEF are degrading to nBM with oral exposure to kill hens at 20 mg/kg/day and higher, then less DEF should be available to produce OPIDN. Less weight was also given to the Abou-Donia *et al.* (1979b) study because of the lack of detail in the published report regarding the incidence and duration of clinical signs, body weight changes, and histopathological lesions. The study also did not meet FIFRA guidelines with respect to the number and age of animals and analysis of the test article. It is difficult to interpret the findings of this study without more information, especially considering the NOAEL is an order of magnitude lower than the NOAELs for any other subchronic neurotoxicity study in hens, including one which met FIFRA guidelines (Sheets, 1991b).

3. Pre- and Post-natal Sensitivity

Developmental toxicity studies in rats and rabbits and reproductive toxicity studies in rats were considered in assessing the potential for higher sensitivity in infants and children than adults. Two developmental toxicity studies were conducted in which DEF was administered by oral gavage, one in rats and the other in rabbits (Kowalski *et al.*, 1986; Clemens *et al.*, 1987). Both studies met FIFRA guidelines. No treatment-related increases in embryotoxicity, fetal malformations or variations were observed in rats and rabbits. Maternal effects included brain ChE inhibition and reduced body weight gain. In rats, the maternal brain ChE activity was reduced (54% of controls) at 28 mg/kg/day on day 20 of gestation; however, fetal brain ChE activity was unaffected. Reductions in the average maternal body weight gain were observed in rats and rabbits at 28 and 9 mg/kg/day, respectively, without corresponding reductions in fetal body weights. These findings in rats and rabbits suggest there is no increased prenatal sensitivity to DEF.

One reproductive toxicity study was available in which DEF was administered in the feed to rats (Eigenberg, 1991a). The study met FIFRA guidelines. Several reproductive effects were seen in this study. The reproductive effects included reductions in the fertility, birth, and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, brain ChE inhibition and gross pathological lesions in pups. The reproductive NOAEL was 32 ppm (3.0 mg/kg/day). Other non-reproductive effects in the adults included brain ChE inhibition and body weight reductions. A reduction in mean brain ChE activity was observed in females (71% of controls) at 32 ppm on day 21 of lactation. No reduction in brain ChE activity was observed in the 21-day-old pups at 32 ppm. At 260 ppm, the reduction in the mean brain ChE activity was significantly greater in adult females (F_0&F_1: 19% of controls) than the 21-day-old pups (F_{2a}: 85% of controls). The mean body weight reductions in the adult females (24%) was similar to the reductions in the 21-day-old pups (25%) at 260 ppm. Based on these findings in rats, there does not appear to be any increased postnatal sensitivity to DEF.

4. Chronic Toxicity

No chronic inhalation toxicity studies were available for DEF. Several adverse effects were seen in oral chronic toxicity studies for DEF (Table 17). Reduced weight gain was

Table 17. Chronic Adverse Effects of DEF and Their Respective NOAELs and LOAELs

Species	Exposure	Effect	NOAEL (mg/kg/day)	LOAEL	Ref.[a]
Mouse	Diet, 90 weeks	Vacuolar degeneration in small intestine, hematopoiesis in spleen, hematological changes (F), brain ChE[b] inhibition (M: 87%)	1.5	8.4	1*
Rat	Diet, 2 years	Liver cytoplasmic vacuolation, reduced weight gain, brain ChE inhibition (F: 69%)	1.25	5.0	2
Rat	Diet, 2 years	Mucosal hyperplasia and vacuolar degeneration in small intestine, hematological changes	0.2	1.8	3*
Dog	Diet, 1 year	Hematological changes (F)	0.4	2.0	4*

a References: 1. Hayes, 1989; 2. Root *et al.*, 1967; 3. Christenson, 1992; 4. Christenson, 1991.
b ChE = cholinesterase. Inhibition expressed as percent of control activity.
* Acceptable study based on FIFRA guidelines

observed in two rat chronic toxicity studies at 100 ppm (5 mg/kg/day) and 320 ppm (M: 16.8 mg/kg/day; F: 21.1 mg/kg/day) (Root *et al.*, 1967; Christenson, 1992). The mean brain ChE activity was reduced in males of the mouse oncogenicity study at 50 ppm (8.4 mg/kg/day - 87% of controls), and in two rat chronic feeding studies at 100 ppm (F: 5 mg/kg/day - 69% of controls) and 320 ppm (M: 16.8 mg/kg/day - 40% of controls; F: 21.1 mg/kg/day - 32% of controls) (Hayes, 1989; Root *et al.*, 1967; Christenson, 1992). The mean brain ChE activity was also significantly reduced (91% of controls) in male mice at 10 ppm (1.5 mg/kg/day). However, this minor reduction was not considered toxicologically significant because no cholinergic signs were observed at 50 ppm and only mild signs (loose stools and perianal stains) were observed at 250 ppm. Hematological changes (reduced RBCs, hemoglobin and hematocrits) were seen in female mice at 50 ppm (11.3 mg/kg/day), in rats of both sexes at 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively), and in female dogs at 64 ppm (2.0 mg/kg/day) (Hayes, 1989; Christenson, 1991; Christenson, 1992). Transient hypothermia was also observed in one rat chronic toxicity study at 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively) (Christenson, 1992).

There were dose-related increases in microscopic lesions in several studies. In a mouse dietary oncogenicity study, an increase in non-neoplastic lesions in the gastrointestinal tract (small intestine vacuolar degeneration, dilated/distended small intestine and cecum, and rectal

necrosis/ulceration), liver (hypertrophy), adrenal glands (degeneration/pigmentation), and spleen (hematopoiesis) was observed at 250 ppm (M: 48.1 mg/kg/day; F: 63.1 mg/kg/day) (Hayes, 1989). The incidence of small intestine vacuolar degeneration and spleen hematopoiesis was also significantly higher in mice at 50 ppm (M: 8.4 mg/kg/day; F: 11.3 mg/kg/day). Increases in several pre-neoplastic lesions in the small intestine of both sexes (mucosal hyperplasia and focal atypia) and the lungs of females (epithelialization and focal hyperplasia) also occurred at 250 ppm (M: 48.1 mg/kg/day; F: 63.1 mg/kg/day). Liver cytoplasmic vacuolation was observed in a rat chronic feeding study at 100 ppm (5 mg/kg/day) (Root *et al.*, 1967). Numerous ocular effects were seen in another rat chronic feeding at 320 ppm (M: 16.8 mg/kg/day; F: 21.1 mg/kg/day) including corneal opacity, lens opacity, cataracts, corneal neovascularization, iritis, uveitis, bilateral flat ERG responses, bilateral retinal atrophy, and optical nerve atrophy (Christenson, 1992). In addition, increased adrenal weights and adrenal vacuolar degeneration were observed in rats at 320 ppm. Vacuolar degeneration of the small intestine were seen in animals exposed for one or two years at both 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively). Mucosal hyperplasia was also observed at 40 and 320 ppm, but only in animals exposed for two years.

The most sensitive endpoint with chronic oral exposure to DEF appears to be the hematological effects. There is evidence in hens that these hematological effects may be due to nBM which inhibits glucose-6-phosphate dehydrogenase (Abdo *et al.*, 1983b). In previous experiments, these investigators isolated nBM in the plasma and excreta of hens and proposed that it is the product of hydrolysis of DEF in the gut (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators also found nBM in the plasma of hens administered DEF dermally, although the concentration was an order of magnitude lower than when the same dose was given orally (Abou-Donia *et al.*, 1979a). Although the hematological changes may be more prevalent with the oral route of exposure, they do not appear to be unique to this route of exposure. nBM is an anticipated metabolite of DEF through its normal metabolism in the liver of animals. Also, DEF readily degrades to nBM in the environment. Slight reductions in erythrocyte counts, hematocrits, and hemoglobin values were seen in a 90-day inhalation study in rats (Pauluhn, 1992). The significance of these hematological effects in assessing the long-term human health risks from exposure to DEF in ambient air is uncertain because exposure is clearly seasonal and these hematological effects appear to be reversible. In the chronic feeding study in rats, some of the hematological values began to return to normal even with continued exposure (Christenson, 1992). In fact, the erythrocyte count, hemoglobin and hematocrit values were higher in the high-dose animals than controls by the end of the 2-year exposure period.

The relevance of the histological lesions in the small intestine, liver and spleen of animals after chronic oral exposure to DEF is also uncertain because similar histological changes were not observed in a 90-day inhalation in rats where these tissues were also examined microscopically (Pauluhn, 1992). The lack of concordance in the histological findings between the subchronic and chronic studies could be due to the difference in either the duration or the route of exposure. Some of these histological lesions, like the vacuolar degeneration and mucosal hyperplasia in the small intestine, could be due to local irritation, from DEF or nBM, and may only be relevant for the oral route of exposure. Therefore, a chronic NOAEL was not selected for DEF because the effects observed in the chronic studies were either previously addressed under seasonal exposure (brain ChE inhibition, hematological changes, lesions in the adrenal glands and eyes) or were not considered relevant to the human exposure scenario because of the duration or route of exposure (lesions in the small intestine, liver and spleen).

5. Oncogenicity

a. Weight of Evidence

There was no evidence of genotoxicity in the four available studies for DEF (an Ames assay, an *in vitro* chromosomal aberrations assay, an *in vitro* sister chromatid exchange assay, and an unscheduled DNA synthesis assay). All of these genotoxicity studies met FIFRA guidelines, except the sister chromatid exchange assay which was a published report.

There was no evidence of oncogenicity in a rat study where DEF was administered in the feed for 2 years (Christenson, 1992). However, an increase in adenocarcinomas of the small intestine (both sexes), liver hemangiosarcomas (males only), and alveolar/bronchiolar adenomas (females only) was seen in mice fed DEF for 90 weeks (Tables 9 and 10) (Hayes, 1989). The adenocarcinomas were often associated with vacuolar degeneration, mucosal hyperplasia and/or focal atypia of the small intestine. The liver hemangiosarcomas were often associated with hemorrhage and necrosis. The increase in alveolar/bronchiolar adenomas were often associated with epithelization and focal hyperplasia. Both oncogenicity studies met FIFRA guidelines.

Although the increase in small intestine adenocarcinomas and alveolar/ bronchiolar adenomas only occurred on at the highest dose level where there was evidence of excessive toxicity, there was insufficient data for DEF to indicate whether any threshold mechanisms might be responsible for the oncogenic response. Moreover, multiple tumor sites were involved, one of which is a rare tumor type (small intestine adenocarcinoma) with a reported historical control range for this laboratory of 0% in both sexes. Consequently, it was assumed there was no

threshold and the potential oncogenic risk to humans was evaluated using a linear, low dose extrapolation model to estimate potency.

b. Quantitative Assessment

It was not possible to accurately estimate the oncogenic potency (Q_1) or upper bound on the slope (Q_1*) for small intestine adenocarcinomas and alveolar/bronchiolar adenomas because the slope estimate is zero when the tumor incidence is only increased at the high dose. Therefore, the incidence of liver hemangiosarcomas in male mice was used to calculate the oncogenic potency of DEF. Due to the reduced survival of mice at the highest dose tested, 250 ppm, the oncogenic potency of DEF was estimated using the multistage-Weibull time-to-tumor model, MULTI-WEIB. The dosages for male mice (0, 1.5, 8.4 or 48.1 mg/kg/day) were converted to human equivalent dosages multiplying by a interspecies scaling factor of body weight to the 3/4 power [$(BWt_A/BWt_H)^{0.25} = (0.030 \text{ kg}/70 \text{ kg})^{0.25} = 0.144$]. The estimated oncogenic potency ranged from 3.3×10^{-2} (maximum likelihood estimate or MLE) to 5.9×10^{-2} (95% upper bound or 95% UB) $(\text{mg/kg/day})^{-1}$. The estimated oncogenic potency for DEF expressed as unit risk is shown in Table 18 relative to other chemicals for which there are oncogenic potency estimates that have been approved by the SRP for TACs. The unit risk estimate for DEF ranged from 9.2×10^{-6} $(\mu g/m^3)^{-1}$ for the MLE to 1.6×10^{-5} $(\mu g/m^3)^{-1}$ for the 95% UB.

C. Exposure Assessment

For more detailed information on the studies used for the exposure estimates, see Part B, Exposure Assessment, of the Evaluation of DEF as a TAC.

1. Offsite Air Exposure

All of the air monitoring data collected in the studies described as off-site under Part A (Section D.1.) were within the ½ mile (# 800 meters) buffer zone. Consequently, no exposure dosages were calculated since people should only be in the buffer zone for short periods of time (< 8 hrs).

Table 18. Oncogenic Potency for DEF Relative to Other Oncogenic Potencies Approved by the Scientific Review Panel for Toxic Air Contaminants

Compound	Unit Risk $(\mu g/m^3)^{-1}$	Range $(\mu g/m^3)^{-1}$
Dioxins	3.8×10^{1}	2.4×10^{1} to 3.8×10^{1}
Chromium IV	1.5×10^{-1}	1.2×10^{-2} to 1.5×10^{-1}
Cadmium	4.2×10^{-3}	2.0×10^{-3} to 1.2×10^{-2}
Inorganic Arsenic	3.3×10^{-3}	6.3×10^{-4} to 1.3×10^{-2}
Benzo[a]pyrene	1.1×10^{-3}	1.1×10^{-3} to 3.3×10^{-3}
Diesel Exhaust	3×10^{-4}	1.3×10^{-4} to 2.4×10^{-3}
Nickel	2.6×10^{-4}	2.1×10^{-4} to 3.7×10^{-3}
1,3-Butadiene	1.7×10^{-4}	4.4×10^{-6} to 3.6×10^{-4}
Ethylene Oxide	8.8×10^{-5}	6.1×10^{-5} to 8.8×10^{-5}
Vinyl Chloride	7.8×10^{-5}	9.8×10^{-6} to 7.8×10^{-5}
Ethylene Dibromide	7.1×10^{-5}	1.3×10^{-5} to 7.1×10^{-5}
Carbon Tetrachloride	4.2×10^{-5}	1.0×10^{-5} to 4.2×10^{-5}
Benzene	2.9×10^{-5}	7.5×10^{-6} to 5.3×10^{-5}
Ethylene Dichloride	2.2×10^{-5}	1.3×10^{-5} to x 2.2×10^{-5}
DEF	**1.6×10^{-5}**	**9.2×10^{-6} to 1.6×10^{-5}**
Inorganic Lead	1.2×10^{-5}	1.2×10^{-5} to 6.5×10^{-5}
Perchloroethylene	5.9×10^{-6}	3.0×10^{-7} to 1.1×10^{-5}
Formaldehyde	6.0×10^{-6}	2.5×10^{-7} to 3.3×10^{-5}
Chloroform	5.3×10^{-6}	6.0×10^{-7} to 2.0×10^{-5}
Acetaldehyde	2.7×10^{-6}	9.7×10^{-7} to 2.7×10^{-5}
Trichloroethylene	2.0×10^{-6}	8.0×10^{-7} to 1.0×10^{-5}
Methylene Chloride	1.0×10^{-6}	3.0×10^{-7} to 3.0×10^{-6}
Asbestos	1.9×10^{-4} (per 100 fiber/m^3)	Lung : 11- 110 x 10^{-6} (per 100 fiber/m^3) Mesothelioma: 38-190 x 10^{-6} per 100 fiber/m^3)

2. Ambient Air Exposure

a. DEF

Exposure dosages were calculated from air monitoring data collected in two different cotton growing regions in the San Joaquin Valley. In a study conducted by Seiber *et al.* (1988) air monitoring data was collected at four different rural locations near Fresno. The air samples collected in this study could be within the buffer zone (10 to 400 meters from cotton fields), but it was not known if the adjacent cotton fields to these sites were treated with DEF. Since there was a school (San Joaquin) and a day center (Huron) in residential settings at two of these sites, they should be outside the buffer zone. The other two monitoring sites (Five Points and Tranquility) may be within the buffer zone since they were not in residential areas nor were there schools at these locations. The Five Points location had not only the highest single daily air concentration for DEF (548 ng/m^3), but it also had the highest mean air concentration for DEF (182 ng/m^3) during this cotton defoliation season. Although this site may be within the buffer zone, the risk estimates were initially calculated for this location, assuming that if the levels were acceptable at this location, they would be acceptable at the other three locations where air concentrations were lower. The estimated exposure levels for children and adults (male and female) are summarized in Table 19. The absorbed daily dosage (ADD) represents an estimate of the highest potential single daily exposure for most people by using the 95th percentile of the exposure during this season. The ADDs ranged from 94.2 to 303.5 ng/kg. The highest exposure estimates were for children due to their higher inhalation rate relative to their body weight. The seasonal average daily dosage (SADD) represents the average exposure during the cotton defoliant season which was assumed to be 60 days. The SADDs ranged from 38.1 ng/kg/day for adult females to 122.7 ng/kg/day for children. The annual average daily dosage (AADD) is the SADD averaged over the year (i.e., SADD multiplied by 60 days, then divided by 365 days). No further adjustments to the AADD were used to evaluate the risk for oncogenicity since an individual could theoretically be exposed his/her entire life by living in the same location. The AADDs ranged from 6.3 ng/kg/day for adult females to 20.2 ng/kg/day for children.

The exact location of the air monitoring sites in relation to cotton fields was not described in the study by Kilgore *et al.* (1984). The locations were described as urban areas in Kern County, although the locations in Bakersfield are probably the only real urban areas. The remaining six locations appear to be rural. The assumption was made that these locations were well outside the buffer zone of any cotton fields. Again, risk estimates were initially calculated for the rural locations with the assumption that if these were acceptable, then the urban

Table 19. Estimated Ambient Air Exposure to DEF for the General Public

	ADD[a] (ng/kg)		SADD[b] (ng/kg/day)		AADD[c] (ng/kg/day)	
	Fresno	Kern	Fresno	Kern	Fresno	Kern
Child	303.5	52.2	122.7	22.8	20.2	3.7
Adult Male	125.8	21.7	50.8	9.4	8.4	1.6
Adult Female	94.2	16.2	38.1	7.1	6.3	1.2

a ADD = Absorbed Daily Dosage. For explanation of the calculations for the exposure estimates, see Tables 5 and 6 in Part B, Exposure Assessment, of the report for DEF as a TAC.

b SADD = Seasonal Average Daily Dosage

c AADD = Annual Average Daily Dosage

areas would be, too. The air samples were only collected on two occasions at these locations, once during the peak application season for DEF and then a second time one week later. Since so few samples were taken from each location, the air concentrations from all six rural locations were combined. The estimated ambient air exposure for children and adults are also summarized in Table 19. Again, children had the highest exposure estimates. The ADDs ranged from 16.2 to 52.2 ng/kg/day, the SADDs from 7.1 to 22.8 ng/kg/day, and the AADDs from 1.2 to 3.7 ng/kg/day.

b. n-Butyl Mercaptan

There was only one study available with air monitoring data for nBM which reported air concentrations of nBM that were one to two orders of magnitude greater than those reported for DEF (CDFA, 1981). However, only a brief summary was available which lacked information about the minimum detection limit, sampling media, efficiency of sampling media, and location of monitoring sites in relation to application sites. Consequently, an exposure assessment was not estimated for nBM due to the lack of confidence in how the air monitoring data were collected in this study.

D. Risk Characterization

1. Acute Toxicity

The risk for non-oncogenic human health effects is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOAEL from experimental animal studies to the human exposure dosage.

$$Margin\ of\ Exposure\ ' \frac{NOAEL}{Exposure\ Dosage}$$

The MOEs for acute exposure to DEF in ambient air were calculated using the ADD for the exposure dosage and the acute NOAELs for blood ChE inhibition (0.6 mg/kg) or clinical signs (2.9 mg/kg). The estimated acute MOEs are summarized in Table 20. Using the NOAEL for blood ChE inhibition, the acute MOEs for ambient air are 2,000 or higher for children and adults. Using the NOAEL for clinical signs, the acute MOEs are all greater than 9,000 for children and adults.

2. Subchronic Toxicity

The estimated seasonal MOEs for DEF are summarized in Table 20. The MOEs for seasonal exposure to DEF in ambient air were calculated using the SADD for exposure dosage and the subchronic NOAELs for blood ChE inhibition (0.6 mg/kg/day) or other effects (2.9 mg/kg/day). Using the NOAEL for blood ChE inhibition, the seasonal MOEs for ambient air exposure are all greater than 4,000. Using the NOAEL for other subchronic effects, the seasonal MOEs are greater than 20,000.

3. Oncogenicity

The risk for oncogenic effects was calculated by multiplying the oncogenic potency by the exposure dosage.

$$Oncogenic\ Risk\ ' \ Oncogenic\ Potency\ x\ Exposure\ Dosage$$

The oncogenic risk was calculated using the highest estimated AADD for an adult for ambient air (8.4 ng/kg/day). The estimated oncogenic potency of DEF based on the incidence of liver hemangiosarcomas in male mice ranged from 3.3×10^{-2} (MLE) to 5.9×10^{-2} (95% UB)

Table 20. Estimated Margins of Exposure for the General Public from Ambient Air Exposure to DEF[a]

		Acute		Seasonal	
	County	Blood ChE[b]	Signs[c]	Blood ChE[d]	Other[e]
Child	Fresno	2,000	9,600	4,900	24,000
	Kern	11,000	56,000	26,000	130,000
Adult Male	Fresno	4,800	23,000	12,000	57,000
	Kern	28,000	130,000	64,000	310,000
Adult Female	Fresno	6,400	31,000	16,000	76,000
	Kern	37,000	180,000	85,000	410,000

[a] Margin of Exposure = NOAEL / Exposure Dosage. Exposure dosages from Table 19. All margins of exposure are rounded to two significant digits.

[b] The acute NOAEL for blood ChE inhibition in rats was 2.4 mg/m^3 (0.6 mg/kg)

[c] The critical NOAEL for acute effects in rats (clinical signs: reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, increased startle response) was 12.2 mg/m^3 (2.9 mg/kg).

[d] The subchronic NOAEL for blood ChE inhibition was 2.4 mg/m^3 (0.6 mg/kg/day).

[e] The critical NOAEL for subchronic effects (clinical signs, brain ChE inhibition, impaired retinal function, pale retinal fundus, fatty droplets in the adrenals, increased adrenal weights) was 12.2 mg/m^3 (2.9 mg/kg/day).

(mg/kg/day)$^{-1}$. To correct for oral absorption, the oncogenic potency was divided by 70%. The resultant adjusted oncogenic potency ranged from 4.7 x 10^{-2} (MLE) to 8.4 x 10^{-2} (95% UB) (mg/kg/day)$^{-1}$. The estimated oncogenic risk from lifetime exposure to ambient air in Fresno county ranged from 3.9 x 10^{-7} (MLE) to 7.1 x 10^{-7} (95% UB). The estimated oncogenic risk from lifetime exposure to ambient air in Kern county ranged from 7.5 x 10^{-8} (MLE) to 1.3 x 10^{-7} (95% UB).

4. n-Butyl Mercaptan

MOEs were not calculated for nBM because of a lack of reliable toxicity and air monitoring data. However, the survey conducted by California Department of Health Services (CDHS) in three communities in cotton-growing regions of California suggest that the air levels of nBM may still be too high based on symptoms that appear to be related to exposure to nBM

(ocular irritation, rhinitis, throat irritation, shortness of breath, wheezing, and asthma-like symptoms) (Scarborough *et al.*, 1989). The American Conference of Government Industrial Hygienists (ACGIH) threshold limit value (TLV) for nBM is 0.5 ppm (ACGIH, 1986). The TLV is based on a study with ethyl mercaptan in which human volunteers were exposed for 3 hours daily. No complaints were recorded at 1 mg/m^3 (0.4 ppm). A reference exposure level (REL) for nBM could also be estimated by dividing the NOAEL of 10 ppm (17 mg/kg/day) from the inhalation developmental toxicity study in mice by an uncertainty factor of 100 for interspecies and intraspecies variation in susceptibility. The estimated REL for nBM is 250 $\mu g/m^3$ or 67.8 ppb, assuming a 24-hr respiratory rate of 0.68 m^3/kg/day for a 6-year-old child. The highest daily average air concentration for nBM (28.6 $\mu g/m^3$ or 7.75 ppb) was reported in the CDFA (1981) study. Therefore, this air concentration is more than 8-fold below the estimated reference exposure level for nBM. However, this air concentration is above the reported odor threshold (0.01 to 1.0 ppb) for nBM (Santodonato *et al.*, 1985). Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms including exacerbating an underlying medical condition, innate odor aversion, odor-related aversive conditioning, stress-induced illness, and possible innate pheromonal reaction (Shusterman, 1992). Ames and Stratton (1991) found that symptoms more closely correlated with odor perception than distance from a potato field treated with ethoprop which breaks down to n-propyl mercaptan.

E. Risk Appraisal

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects observed in toxicity studies with laboratory animals will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for DEF are delineated in the following discussion.

1. Hazard Identification

The metabolism of DEF by the various routes of exposure is uncertain since only a few metabolites have been identified. DEF sulfoxide and S,S-dibutyl-S-1-hydroxybutyl

phosphorotrithioate were identified in rat urine after intraperitoneal injection of DEF (Hur *et al.*, 1992). A number of metabolites were detected in the urine and feces of several species (rat, goat, chicken) after oral administration of DEF; however, only one metabolite, butyl-gamma-glutamylcysteinylglycine, was identified in rat urine (Kao *et al.*, 1991; Hall, 1991; Sahali, 1991). These investigators suggested that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. nBM was also identified in the excreta of hens administered DEF orally (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators proposed that DEF was hydrolyzed to nBM in the gut causing the late acute effects which were only observed with oral administration of DEF. The hydrolysis of DEF in the gut could be due to either simple degradation or microbial metabolism. Due to the differences in the gastrointestinal tract between birds and mammals, it is unknown if DEF is also easily hydrolyzed to nBM in the gut of mammals. Clinical signs similar to late acute effects in hens have not been observed in mammals; however, similar hematological effects have been observed in chronic feeding studies in mice, rats, and dogs. These hematological changes and the gastrointestinal lesions observed in the chronic feeding studies may be route-specific effects due to nBM rather than DEF. Consequently, these endpoints may not be relevant for occupational exposure in humans which occurs primarily by the dermal route. Although metabolic pathways were proposed based on these few metabolites, the metabolism of DEF by the various routes is still highly speculative.

The physiological role of AChE in the nervous system is well known; however, there is some uncertainty regarding the toxicological significance of brain ChE inhibition because of the poor correlation between the severity of cholinergic signs and the level of ChE inhibition in the brain (U.S. EPA, 1988b). Several factors probably contribute to the poor correlation. One of these factors is that ChE inhibitors produce different degrees of inhibition in the various regions of the brain (Nieminen *et al.*, 1990). Another factor is that some cholinergic signs may be due to peripheral rather than central inhibition of AChE (Murphy, 1986). In addition, brain ChE activity is usually measured at the end of the study whereas the cholinergic signs may be observed at various time points during the study. Often cholinergic signs are observed only at the beginning of the study and then the animals appear to develop a "tolerance" to the ChE inhibitor. This adaptation or "tolerance" may be due to several possible mechanisms including “down-regulation” or reduction in the number of post-synaptic receptors (Costa *et al.*, 1982). Finally, clinical observation in animal studies is a very crude and subjective measurement. Some mild cholinergic signs, such as headaches and anxiety, cannot readily be detected in animals. There may also be other subtle changes in neurological function that will only be detected if the animal is stressed or required to perform certain tasks (Nagymajtényi *et al.*, 1988; Raffaele and Rees, 1990). It is possible that some level of brain ChE inhibition can occur without any untoward effect on

neurological function, overt or subtle. However, in the absence of rigorous behavioral and neurophysiological testing, the assumption is made that if there is statistically significant inhibition of brain ChE inhibition, there is probably some deleterious effect to the nervous system.

The critical NOAEL of 12.2 mg/m^3 (2.9 mg/kg) selected by DPR toxicologists for evaluating acute exposure to DEF in ambient air was based on clinical signs (reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, and increased startle response) observed in rats during the first 3 days of exposure in a 90-day inhalation study (Pauluhn, 1992). While the clinical signs were not clearly cholinergic in origin, they appear to be treatment-related because these signs were only observed at the highest dose, 59.5 mg/m^3 (14.3 mg/kg). Furthermore, similar effects were observed in two inhalation LC_{50} studies in rats (Thyssen, 1978a; Warren, 1990). Since most of these signs were not observed until more than one dose was administered, it is possible that the NOAEL after a single day of exposure would be higher. However, the general public is more likely to be exposed to DEF in ambient air for more than one day. A NOAEL of 77 mg/m^3 (12.3 mg/kg/day) was observed in an inhalation LC_{50} study conducted by Thyssen (1978a). This study was not selected because of major deficiencies in the study, the most significant being no summary of clinical signs by dose group. If this NOAEL has been selected, the acute MOEs would be approximately 4 times greater than estimated. A NOAEL was not observed in another inhalation LC_{50} study which met FIFRA guidelines (Warren, 1990). A NOAEL of 159 mg/m^3 (25.4 mg/kg) could be estimated by dividing the LOAEL by the uncertainty factor of 10. If this estimated NOAEL had been used, the acute MOEs would be approximately 9 times larger than estimated. However, a slightly larger uncertainty factor, such as 30, may be more appropriate to use in this case because death was observed at the LOAEL. If a lower estimated NOAEL of 53 mg/m^3 (8.5 mg/kg) had been used, the acute MOEs would only be 3 times larger than estimated.

The critical NOAEL of 12.2 mg/m^3 (2.9 mg/kg/day) was also selected by DPR toxicologists for evaluating seasonal exposure to DEF in ambient air based on the clinical signs, brain ChE inhibition (60% of controls), impaired retinal function, hematological changes, pale retinal fundus, increased adrenal weights, and fatty droplets in the adrenal cortex (Pauluhn, 1992). Slightly lower NOAELs were observed in two oral studies. In a rat reproductive toxicity study, a NOAEL of 0.4 mg/kg/day was observed based on brain ChE inhibition (71% of controls) in adult females. This study was not used because of the route of exposure and the uncertainty regarding the impact of the increased DEF intake in the females during lactation on the reduced brain ChE activity at terminal sacrifice. However, if this NOAEL had been used for evaluating seasonal exposure to DEF in ambient air, the MOEs would be approximately 7 times lower than estimated. Since the DEF intake in females was higher during lactation just prior to their terminal sacrifice, it

may be more appropriate to use the average compound consumption during lactation (0.7 mg/kg/day) as the NOAEL for females rather than the time-weighted average (0.4 mg/kg/day). If the DEF intake during lactation was used as the critical NOAEL, the seasonal MOEs would be only 4 times lower than estimated.

A NOAEL of 0.1 mg/kg/day was observed in a 90-day oral neurotoxicity study in hens (Abou-Donia *et al.*, 1979b). This study was not used for a variety of reasons as previously discussed under the Hazard Identification section, the most significant being the uncertainty about the relevance of the mild ataxia observed at the LOAEL because of the route of exposure. It is very possible that the mild ataxia is due to nBM (which also causes incoordination) rather than DEF since unequivocal evidence of OPIDN (paralysis and nerve degeneration) were not observed in the hens until 20 mg/kg/day. These same investigators had proposed that DEF is hydrolyzed in the gastrointestinal tract to nBM. If the ataxia is caused by nBM, then this effect is not necessarily relevant to human exposure to DEF in ambient air. However, if the NOAEL from this study had been used to evaluate seasonal exposure, the MOEs would be approximately 30 times lower than estimated (i.e., > 800).

A NOAEL was not selected for any of the effects observed in chronic feeding studies with animals either because these effects (brain ChE inhibition, hematological effects, adrenal and ocular lesions) were already addressed under subchronic toxicity or because they were not considered relevant to human exposure to DEF in ambient air due to differences in duration or route of exposure (lesions in the small intestine, liver and spleen). If MOEs had been calculated to evaluate chronic exposure using the lowest NOAEL from the chronic studies, 0.2 mg/kg/day, they would be approximately 2.5 times lower than the seasonal MOEs.

There was a significant increase in the incidence of adenocarcinomas in the small intestine of both sexes, in liver hemangiosarcomas in males, and alveolar/bronchiolar adenomas in females in a mouse study; however, there was no evidence of an oncogenic effect in a rat oncogenicity study and the genotoxicity data was negative. Among the tumors seen in mice only the liver hemangiosarcomas in males had an increase in the incidence at doses below the highest dose tested. There was also a significant increase in marked anemia, non-neoplastic degenerative lesions in the gastrointestinal tract and adrenal gland, and non-oncogenic mortality (females) at the high dose, 250 ppm, suggesting the maximum tolerated dose (MTD) had been exceeded. At or above the MTD, normal physiology, metabolism and/or repair mechanisms may be overwhelmed, resulting in the initiation or promotion of tumors (Carr and Kolbye, 1991; Swenberg, 1995). Increased cell proliferation due to cytotoxicity can result in the promotion of tumors by decreasing the time available to repair DNA damage. Other nongenotoxic mechanisms,

such as immunosuppression or endocrine disruption, could also be responsible for the increase in tumors (MacDonald *et al.*, 1994). If a threshold mechanism, such as increased cell proliferation was involved, the use of a linearized multistage model to estimate oncogenic risk would exaggerate the risk since it assumes there is no biological threshold.

Very little is known about the toxicity of nBM. Only a few studies were available describing the effects in laboratory animals after acute exposure. Some effects observed in animals were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma (Fairchild and Stokinger, 1958). Other effects included restlessness, increased respiration, diarrhea, ocular irritation, liver and kidney damage. Evidence of respiratory irritation was seen with inhalation exposure, including sneezing, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lungs. There was insufficient information available in the published report by Fairchild and Stokinger (1958) to establish a NOAEL by any of the routes tested.

Abou-Donia and coworkers (1979a & 1984) administered single doses of nBM to hens and observed various clinical including malaise, leg or general weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors, loss of breath, and just prior to death, a dark and droopy comb. The NOAEL was 100 mg/kg based on these clinical signs. Abdo *et al*. (1983b) found that hens administered nBM had elevated methemoglobin levels and reduced erythrocyte counts, hematocrit, hemoglobin levels and G-6-PD activity. Because the time course of the hematological changes and the clinical signs were similar, the investigators proposed that the inhibition of G-6-PD was responsible for the hematological changes. A NOAEL was not established for the hematological effects in any of the toxicity studies for nBM.

An acute NOAEL of 10 ppm (17 mg/kg/day) was established in a developmental toxicity study based on increased mortalities, reduced body weight gain, unkempt appearance, lethargy, red/brown stains, increased post-implantation losses and fetal malformations in mice. Complaints of nausea, eye and respiratory irritation among residents of communities in cotton-growing regions have been attributed to nBM, which has a strong skunk-like odor (Maddy and Peoples, 1977; Scarborough, 1989). It is not clear if ocular and respiratory irritation were evaluated in the developmental toxicity study in mice. It also does not appear that the mice were evaluated for hematological changes. Therefore, it is possible the acute NOAEL for nBM would be lower based on these endpoints.

There were no studies available in which animals were exposed to nBM on a subchronic or chronic basis. Consequently, the potential long-term health effects in humans from seasonal or

chronic exposure to nBM are unknown. The long-term health effects from nBM are of particular concern since there is evidence of oncogenicity in mice administered DEF orally. If DEF is significantly hydrolyzed to nBM in the gut of mice as it is in chickens, it is possible that the oncogenicity may be due to the nBM rather than DEF. Additionally, no genotoxicity data were available for nBM either.

2. Exposure Assessment

Most of the uncertainties associated with the exposure assessment have already been discussed in Part B, Exposure Assessment, of the report for DEF as a TAC. However, there are several major uncertainties in the exposure assessment that should be emphasized. In the Seiber *et al.* (1988) study, information regarding the use of DEF on nearby cotton fields was not available. Due to their close proximity (10 to 400 meters) to cotton fields, the assumption was made that they were outside the buffer zone and were considered ambient. The monitoring sites at Five Points and Tranquility may be within the buffer zone because these locations were not zoned residential and there were no schools at these locations. Since the Five Points site may be within the buffer zone, the MOEs for ambient air exposure in Fresno county may be larger than estimated. If the monitoring data from the San Joaquin School site had been used, the MOEs for ambient air exposure in Fresno county would be approximately twice as large as estimated.

A comparison of the ambient air exposures in Fresno and Kern counties is difficult since there was no information regarding the distance of the air sampling sites from cotton fields in Kern county. It is unclear if the lower air concentrations in Kern county were due to climatic differences, the distance from cotton fields or fewer fields being treated.

There was inadequate air monitoring data for nBM to estimate the seasonal and chronic exposure to this degradation product. Seasonal and chronic exposure could not be estimated since the minimum detection limit and the days where nBM was not detected were not reported. The exposure estimates for both DEF and nBM were also limited by the lack of information regarding the inhalation retention and absorption of these compounds. Although the actual retention and absorption is probably less, a 100% retention and absorption were assumed which would result in higher exposure estimates and, thus, would be considered more health protective.

3. Risk Characterization

Generally, a margin of exposure of at least 100 is considered sufficiently protective of human health when data are derived from animal studies. The MOE of 100 allows for humans being 10 times more sensitive than animals and for the most sensitive human being 10 times more sensitive than the average human. The MOEs for acute and seasonal exposure to DEF in ambient air were all greater than 1,000 for both adults and children using either blood ChE inhibition or other effects.

MOEs were not calculated for nBM because of limited toxicity and monitoring data. However, a reference exposure level of 250 $\mu g/m^3$ or 67.8 ppb was calculated for nBM based on a NOAEL from a developmental toxicity study in mice (Thomas *et al.*, 1987). The highest reported daily average air concentration for nBM, 28.6 $\mu g/m^3$ or 7.76 ppb, from a CDFA (1981) study was below the reference level by an order of magnitude. On the other hand, this air concentration was well above the odor threshold for nBM which ranges from 0.01 to 1 ppb. Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms (Shusterman, 1992).

An oncogenic risk level less than 10^{-6} is generally considered negligible. The estimated oncogenic risk from DEF in ambient air were all less than 10^{-6}. In using the Multistage-Weibull model to estimate oncogenic potency, the assumption is that there is no threshold and the response is linear which may have exaggerated the oncogenic risk. On the other hand, if the oncogenic effect is due to nBM, rather than DEF, the risk levels may be underestimated since the air levels of nBM appeared to be several orders of magnitude higher than DEF.

4. U.S. EPA's Reregistration Eligibility Document for DEF

U.S. EPA made available in September 1998 a draft of the Health Effects Division (HED) chapter of the Reregistration Eligibility Document (RED) for tribuphos (DEF) on the Internet for public comment (U.S. EPA, 1998) . U.S. EPA did not estimate exposure to the general public from DEF in the ambient air. However, they did evaluate inhalation exposure in workers using the 90-day inhalation study conducted by Pauluhn (1992) which was selected as the definitive study in DPR's risk assessment addressing acute and seasonal exposure to DEF in ambient air. Although the same study was selected by DPR and U.S. EPA for evaluating inhalation exposure, different critical NOAELs were identified for this study because of different policies these agencies have regarding the use of blood ChE as a regulatory endpoint. Both agencies do not considered blood ChE inhibition to be an adverse effect in itself; however, U.S. EPA uses it as a surrogate for peripheral ChE inhibition data when it is not available which it generally is not. Due to the differences in these policies, U.S. EPA identified a NOAEL of 0.9 mg/kg/day for this 90-day inhalation study based on plasma and erythrocyte ChE inhibition. If DPR had used this NOAEL in calculating the MOEs for DEF in ambient air, the MOEs would have ranged from 3,000 to 55,000 for acute exposure and from 7,300 to 130,000 for seasonal exposure.

U.S. EPA had previously used the Abou-Donia *et al.* (1979b) study to calculate an RfD for DEF when many of the acceptable registrant studies were not available. In this draft RED document, the RfD is no longer estimated using the Abou-Donia *et al.* (1979b) study. Instead, the RfD was calculated using a NOAEL of 0.1 mg/kg/day based on plasma ChE inhibition in dogs fed DEF in the diet for one year (Christenson, 1991). U.S. EPA identified a NOAEL of 11 mg/kg/day for delayed neurotoxicity study in the 90-day neurotoxicity study in hens submitted by the registrant (Sheets, 1991b). This NOAEL is higher than the NOAEL of 2.6 mg/kg/day that DPR toxicologists identified for this study based on delayed neuropathy. DPR toxicologists made the health protective assumption that the slight increase in equivocal lesions at 11 mg/kg/day was treatment-related, even though it was not statistically significant.

As part of the Food Quality Protection Act (FQPA), U.S. EPA evaluated the developmental and reproductive toxicity studies for DEF and concluded, as did DPR toxicologists, that there was no evidence for increased pre- or post-natal sensitivity. However, they recommended that the 10X uncertainty factor for children be retained because of data gaps. The registrant had not submitted acute and subchronic neurotoxicity studies in rats. U.S. EPA was also requesting a developmental neurotoxicity study in rats based on the delayed neurotoxicity and ocular toxicity caused by DEF and a special 90-day study to further evaluate the ocular toxicity. None of these studies are required under the Birth Defect Prevention Act (SB 950) to register pesticides in California.

U.S. EPA classified DEF as a Likely High Dose/Not Likely Low Dose carcinogen under its new carcinogenicity classification system. Their justification for this classification was that tumors were only increased at the highest dose level where severe toxicity occurred. Although not explicitly stated, this classification treats DEF is a threshold carcinogen because they use an MOE approach to protect for oncogenicity, rather than calculate an oncogenic potency factor. The NOAEL they selected to calculate the MOEs for oncogenicity was 0.1 mg/kg/day based on plasma ChE inhibition in dogs, the most sensitive endpoint for chronic exposure (i.e, the same NOAEL used for calculating the RfD). If DPR toxicologists had used this approach in evaluating oncogenic risk from exposure to DEF in ambient air, the MOEs for oncogenicity would have ranged from 12,000 to 83,000.

F. Reference Exposure Levels

Air concentrations of DEF below the reference exposure level are considered sufficiently low to protect human health. The reference exposure levels were calculated by dividing the NOAELs for acute and subchronic chronic effects in animals by an uncertainty factor of 100 to account for interspecies and intraspecies variation in susceptibility (see Appendix A, Equations 3 and 4). The NOAELs were adjusted for differences in the respiratory rate between species. Since children had the highest respiratory rate for humans relative to their body weight, their respiratory rate was used for humans. The adjusted NOAEL for acute and subchronic blood ChE inhibition is 880 $\mu g/m^3$, assuming a 24-hr respiratory rate of 0.68 m^3/kg for a 6-year old child (see Part B, Exposure Assessment, of the report for DEF as a TAC). The resultant reference exposure level (REL) is 8.8 $\mu g/m^3$ (0.68 ppb). The adjusted NOAEL for the other acute and subchronic effects is 4.3 mg/m^3. The REL for DEF based on these other acute and subchronic effects is 43 $\mu g/m^3$ (3.3 ppb). The air concentration which corresponds to a negligible oncogenic risk level (i.e., 10^{-6}) was calculated by first estimating the exposure dosage divided by the 95% UB estimate of oncogenic potency (8.4×10^{-2} $(mg/kg/day)^{-1}$). For DEF, the exposure dosage corresponding to a negligible oncogenic risk is 11.9 ng/kg/day. Assuming a lifetime exposure is necessary to produce the oncogenic effect, the exposure dosage was converted to an air concentration by dividing by the estimated breathing rate for an adult male (0.28 $m^3/kg/day$). The air concentration below which there would be no regulatory concern for oncogenic effects is 42 ng/m^3 (3.3 ppt).

G. Conclusions

The critical NOAELs selected by DPR toxicologists for evaluating potential adverse health effects in humans exposed to DEF on an acute and seasonal basis were both 2.9 mg/kg/day. Although there is no known physiological function for ChE in plasma and erythrocytes, the SRP

for TACs recommended evaluating acute and seasonal exposure to DEF in ambient air using the NOAEL for blood ChE inhibition (0.6 mg/kg/day) due to the possible role of plasma ChE in the metabolism of various drugs. There was sufficient evidence of oncogenic potency to warrant calculation of oncogenic risk by low-dose extrapolation using a linearized multistage model. The estimated oncogenic potency ranged from 4.7×10^{-2} to 8.4×10^{-2} $(mg/kg/day)^{-1}$. Because of their higher respiratory rate relative to their body weight, children consistently had the highest potential exposures. The highest estimated acute and seasonal exposure dosages for DEF in ambient air were 303 and 123 ng/kg/day, respectively, for children at the Five Points location in Fresno county. Using the NOAEL for blood ChE inhibition, the margins of exposure for acute and seasonal exposure for children at the Five Points location were 2,000 and 4,900, respectively. Based on the NOAEL for other effects, the corresponding margins of exposure for acute and seasonal exposure for children at the Five Points location were 9,600 and 24,000, respectively. Generally, a margin of exposure of at least 100 is desirable to account for interspecies and intraspecies variation in susceptibility. The highest estimated oncogenic risk for DEF in ambient air was for the Five Points location which ranged from 3.9×10^{-7} (MLE) to 7.1×10^{-7} (95% UB). An oncogenic risk level less than 10^{-6} is generally considered negligible. MOEs could not be calculated for nBM since only limited toxicity and air monitoring data were available for nBM.

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APPENDICES

APPENDIX A Equations for Inhalation Studies

APPENDIX B Oncogenicity Computer Model Printout

APPENDIX A - EQUATIONS FOR INHALATION STUDIES

1. Dose estimation for animals from an inhalation study when exposure level is in mg/m^3:

$$dose\ (mg/kg/day) = mg/m^3 \times RR_a \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} \times AF$$

2. Dose estimation for animals from an inhalation study when exposure level is in ppm:

$$dose\ (mg/kg/day) = ppm \times \frac{M.Wt.}{M.Vol.} \times RR_a \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} \times AF$$

3. Equivalent 24 hours exposure level for humans by inhalation exposure:

$$human\ equivalent\ exposure\ (mg/m^3) = \frac{dose\ (mg/kg/day)}{RR_h}$$

4. Reference exposure levels from inhalation exposure based on margin of exposure:

$$reference\ exposure\ level\ (mg/m^3) = \frac{human\ equivalent\ exposure\ (mg/m^3)}{margin\ of\ exposure\ (e.g.,100)}$$

$$reference\ exposure\ level\ (ppm) = reference\ exposure\ level\ (mg/m^3) \times \frac{M.Vol.}{M.Wt.}$$

NOTE: 1 mg/m^3 = 1 µg/liter

1 ppm = 1 µg/ml

M.Wt. = molecular weight in grams

M.Vol. = molecular volume which is 24.45 liters at 25°C

RR = respiratory rate in m^3/kg/day where a is for animal and h is for human.

AF = respiratory retention/absorption factor

APPENDIX B

Oncogenicity Computer Model Printout

DATE: 04-05-96 TIME: 09:09:17

MULTI-WEIB (MAR 1985)

K.S. CRUMP & COMPANY, INC.
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

Liver Hemangiosarcomas in Males - Term. Sac. Nonfatal

THE 16 OBSERVATIONS AT LEVEL 1 WITH A DOSE OF .000000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
25.0	1	0	51.0	1	0
53.0	1	0	61.0	1	0
72.0	1	0	76.0	1	0
78.0	1	0	80.0	1	0
84.0	1	0	84.0	1	3
85.0	1	0	87.0	2	0
88.0	1	0	89.0	1	0
90.0	1	0	91.0	34	0

THE 14 OBSERVATIONS AT LEVEL 2 WITH A DOSE OF .220000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
49.0	1	0	51.0	1	0
55.0	1	0	57.0	1	0
60.0	1	0	65.0	1	0
72.0	1	0	79.0	2	0
81.0	1	0	82.0	1	0
83.0	1	0	90.0	2	0
91.0	35	0	91.0	1	2

THE 19 OBSERVATIONS AT LEVEL 3 WITH A DOSE OF 1.21000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
44.0	1	0	47.0	1	0
53.0	1	0	59.0	1	3
64.0	1	0	64.0	1	3
68.0	1	0	69.0	1	0
70.0	1	0	75.0	1	0
80.0	1	0	81.0	1	3
81.0	1	0	83.0	1	0
87.0	3	0	88.0	3	0
89.0	1	0	91.0	28	0
91.0	1	2			

THE 26 OBSERVATIONS AT LEVEL 4 WITH A DOSE OF 6.91000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
25.0	1	0	32.0	1	0
47.0	1	0	52.0	1	0
64.0	1	0	69.0	2	0
70.0	1	0	72.0	1	0
73.0	1	0	74.0	1	0
75.0	2	3	75.0	3	0
76.0	1	0	77.0	1	0
77.0	1	3	78.0	1	0
79.0	2	3	83.0	1	0
85.0	1	0	85.0	1	3
87.0	2	0	88.0	1	0
89.0	1	0	90.0	1	0
91.0	19	0	91.0	1	2

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp((-Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3) * (T - T0)^J
)

THE MAXIMUM LIKELIHOOD ESTIMATION OF:

PROBABILITY FUNCTION COEFFICIENTS

Q(0)= .143009876262E-09
Q(1)= .169936984452E-09
Q(2)= .000000000000
Q(3)= .000000000000

TIME FUNCTION COEFFICIENTS

T0 = 24.9999090000
J = 4.55852108337

THE MAXIMUM LIKELIHOOD IS -31.4923915518

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK
****

WEIBULL LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	LOWER BOUND ON DOSE	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
1.000000E-06	2.987309E-05	1.666645E-05	1.792408E-06	95.0%	91.0000

WEIBULL UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
1.00000	3.292087E-02	5.901792E-02	95.0%	91.0000

NORMAL COMPLETION!